CORRELATION BETWEEN TOTAL LEUCOCYTE COUNT, MICROALBUMINURIA AND CARDIOVASCULAR RISK IN TYPE 2 DIABETES MELLITUS-A CROSS SECTIONAL STUDY

Dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

CHENNAI – 600032

In partial fulfillment of the requirement for the degree of

Doctor of Medicine in Physiology (Branch V)

M.D. (PHYSIOLOGY)

APRIL 2016

DEPARTMENT OF PHYSIOLOGY

TIRUNELVELI MEDICAL COLLEGE

TIRUNELVELI – 11.

CERTIFICATE

This is to certify that the dissertation entitled, **"CORRELATION BETWEEN TOTAL LEUCOCYTE COUNT, MICROALBUMINURIA AND CARDIOVASCULAR RISK IN TYPE 2 DIABETES MELLITUS-A CROSS SECTIONAL STUDY"** by DR. M. PRADEEPA Post graduate in PHYSIOLOGY (2013-2016), is a bonafide research work carried out under our direct supervision and guidance and is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, for M.D. Degree Examination in Physiology (Branch V), to be held in April 2016.

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ENDORSEMENT BY THE GUIDE

This is to certify that the dissertation entitled **"CORRELATION BETWEEN TOTAL LEUCOCYTE COUNT, MICROALBUMINURIA AND CARDIOVASCULAR RISK IN TYPE 2 DIABETES MELLITUS-A CROSS SECTIONAL STUDY"** is a bonafide research work carried out by Dr. M. PRADEEPA in the Department of Physiology, Tirunelveli Medical College Hospital, Tirunelveli -11 under my direct guidance and supervision in partial fulfillment of the requirement for the award of the degree of MD in PHYSIOLOGY (Branch - V) in April 2016.

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DECLARATION

I solemnly declare that the dissertation entitled **"CORRELATION BETWEEN TOTAL LEUCOCYTE COUNT, MICROALBUMINURIA AND CARDIOVASCULAR RISK IN TYPE 2 DIABETES MELLITUS- A CROSS SECTIONAL STUDY"** is done by me at Tirunelveli Medical College Hospital, Tirunelveli.

The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University towards the partial fulfillment of requirements for the award of M.D. Degree (Branch V) in Physiology.

Place : Tirunelveli

Date:

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3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
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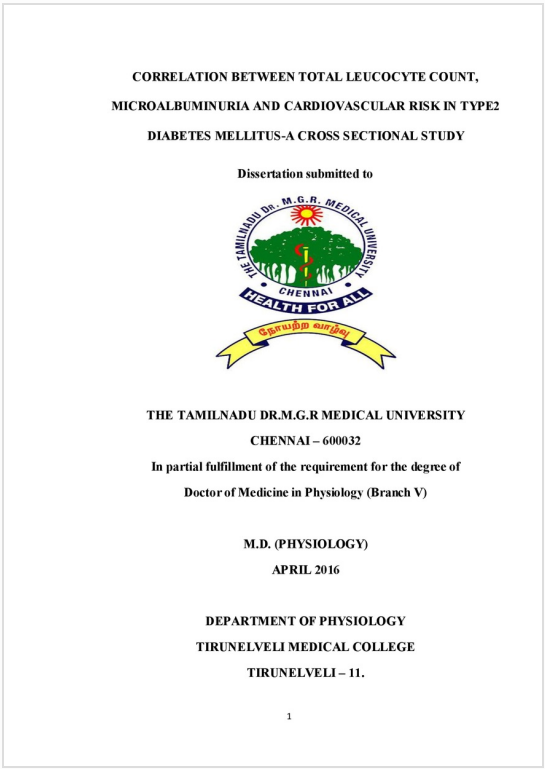

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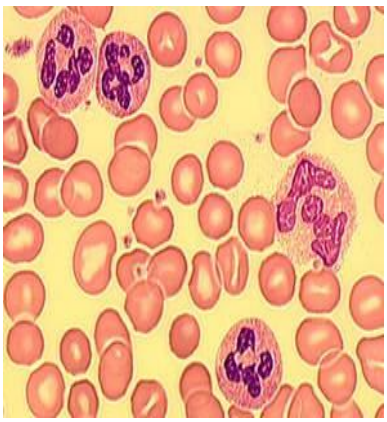

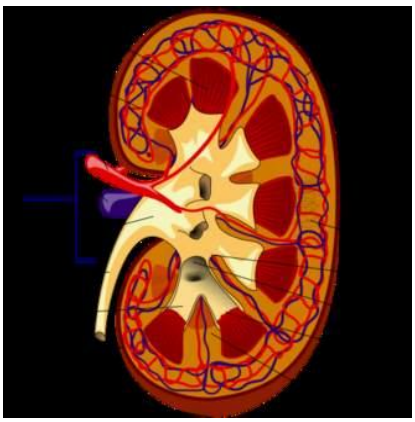

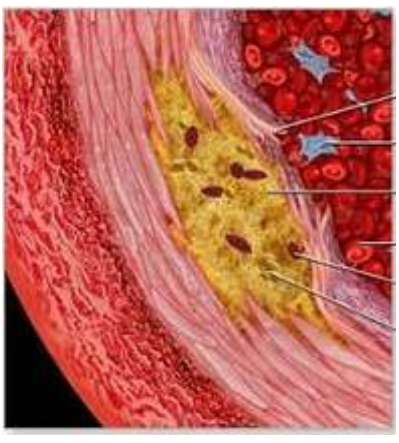

CONTENTS

ABBREVATIONS

WHO	WORLD HEALTH ORGANISATION
IDF	INDIAN DIABETIC FEDERATION
WBC	WHITE BLOOD CELL
TLC	TOTAL LEUCOCYTE COUNT
DC	DIFFERENTIAL COUNT
BMI	BODY MASS INDEX
GLUT	GLUCOSE TRANSPORTER
GLP	GLUCAGON LIKE PEPTIDE
GIP	GLUCOSE DEPENDENT INSULINOTROPIC PEPTIDE
LDL	LOW DENSITY LIPOPROTEIN
HDL	HIGH DENSITY LIPOPROTEIN
IRS	INSULIN RECEPTOR SUBSTRATE
IR	INSULIN RESISTANCE
IGF	INSULIN LIKE GROWTH FACTOR
ACR	ALBUMIN CREATININE RATIO
UAC	URINARY ALBUMIN CONCENTRATION
CRP	C REACTIVE PROTEIN
AGE	ADVANCED GLYCATION END PRODUCTS
ASCVD	ATHEROSCLEROTIC CARDIOVASCULAR DISEASE
SBP	SYSTOLIC BLOOD PRESSURE

ABSTRACT

BACKGROUND: Cardiovascular disease is the leading cause of death in the world. The mortality rate due to cardiovascular risk is six times more pronounced in individuals with diabetes mellitus than in others. So we have to diagnose the cardiovascular risk at an earlier date to prevent the onset of complications. AIMS AND ABJECTIVES: (i) To find the correlation between total leucocyte count, microalbuminuria and cardiovascular risk in type 2 diabetes mellitus. (ii) to evaluate other risk factors of diabetes like increased BMI, waist circumference, family history, and smoking with microalbuminuria, leucocyte count and cardiovascular risk. MATERIALS AND METHODS: After getting institutional ethical commitee200 diabetic individuals of both men and women between ages 35-55 years were selected. Exclusion criteria includes type 1 diabetes mellitus, known malignant disease, after vigorous exercise, history of heart disease, urinary tract infection, liver cirrhosis, haematological diseases, advanced renal dysfunction(serum creatinine >2.0mg/dl), those taking medications like steroid and anti allergy agents. Parameters like blood pressure, waist circumference, BMI, total leucocyte count, fasting blood sugar, HbA_1C, urine for microalbuminuria and ECG changes were recorded in a master chart in two groups microalbuminuria group and normoalbuminuria group. Results analysed using mean, standard deviation, correlation was calculated using spearman rho correlation. RESULTS: The prevalence of microalbuminuria was 33%, increased leucocyte count was found 27%, and ECG changes were found in 24%. There is a significant correlation between microalbuminuria, total leucocyte count. Microalbuminuria was associated with increased blood pressure, smoking, HbA_1C, fasting blood sugar. CONCLUSION: Since there exists a correlation between total leucocyte count, microalbuminuria, and cardiovascular risk, total leucocyte count can also be used as a simple and cost effective method to detect the microvascular and macrovascular complications earlier thereby reducing the mortality and morbidity associated with complications.

Key words: Type 2 diabetes mellitus, microalbuminuria, total leucocyte count, cardiovascular risk

INTRODUCTION

Cardiovascular disease is the leading cause of mortality in the world. According to WHO 2012, nearly 17.5 million deaths per year are due to this condition[1]. Now in developing countries cardiovascular disease has become a major health concern in the middle age population and the cardiovascular mortality is much more than in developed countries. This result in the economic burden of the family due to loss of productivity of working individuals and these cardiovascular changes are more pronounced in individuals with diabetes mellitus than in others.

In 1997, the **American diabetes association** defined diabetes as "a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. **WHO** modified this definition as "a metabolic cum vascular syndrome of multiple etiology characterised by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both leading to changes in small blood vessels (Microangiopathy) and large blood vessels (macroangiopathy)".Two types of diabetes mellitus have been identified type 1 and type 2 diabetes mellitus. Among them 90% is type 2 diabetes and it is underway increasing both in developed and developing countries[2].

Globally the prevalence of diabetes is expected to arise from 220 million in 2010 to 300 million in 2025 and each diabetic individual will spend about three times greater on their health compared to a person without diabetes[3].

Type 2 diabetes mellitus is a chronic progressive disease associated with long term devastating complications which can be fatal and becoming one of the world's most important non communicable diseases. It is known to occur in genetically predisposed persons, exposed to environmental influences that precipitate the onset of clinical disease. Diabetes can be differentiated into monogenic and polygenic forms based on clinical grounds and in terms of genes involved in the pathogenesis of this disorder[4]. Risk factors include age, sex, and ethnic background etc.

Epidemiological determinants and risk factors of type 2 diabetes include family history, genetic markers, thrifty genes and demographical characters like sex, age, ethnicity, behaviour and life style related risk factors like obesity, physical inactivity, diet, stress, westernisation, urbanisation and modernisation[5]. This metabolic disorder results from insulin resistance resulting in various pathophysiological changes in body organs. Previous thought was that children and young adults were affected by type 1 diabetes and type 2 diabetes occurs in middle aged individuals. But this has started changing now because of increased prevalence of diabetes.

As the age of onset of diabetes has decreased and average lifespan of an individual has increased, they are more prone to develop complications and have to live longer with complications.[6] Most of the morbidity and mortality of diabetes is due to the chronic complications of the disease. The chronic complications of diabetes are due to pathological changes affecting blood vessels of the involved organs. The disease can involve either small blood

vessels or larger blood vessels. Diabetic retinopathy and nephropathy are predominantly microvascular diseases and cardiovascular disease occurs due to macrovascular changes.

These complications can be reduced by educating the patient about the disease, its complications and by regular follow up investigations. Poor glycemic control that result in long term complications leads to morbidity, mortality and economic burden of the disease.

The basic pathology of macrovascular disease is atherosclerosis. Risk factors for atherosclerosis in diabetes mellitus include hyperglycemia, hyperlipidemia, hypertension, cigarette smoking, and positive family history of diabetes[7]. There is an increasing evidence of association between fasting blood glucose and risk of macrovascular complications.

Cardiovascular complications are increased by two fold to six fold in individuals with diabetes. The mortality rate is increased in diabetic individuals than normal persons because of diabetic complications. There is a continuous relationship between level of glycemia and risk of development of these complications. Hyperglycemia and insulin resistance appears to play an important role in the development of complications.

Peripheral leucocyte count is associated with insulin resistance, type 2 diabetes, coronary vascular disease, stroke and diabetes related micro and macrovascular disease. There are studies indicating that an elevated WBC count even within the normal range is associated with complications in type 2 diabetes. An association between leucocyte count and coronary artery disease

has been observed after adjusting for multiple risk factors of coronary heart disease including smoking[8].

And there is upcoming evidence that leucocyte count, fibrinogen and C-reactive protein are all positively correlated with atherosclerosis accompanied by inflammation. There have been only few researches on the relation between vascular complications and leucocyte count. Although studies are available for relation between microalbuminuria and cardiovascular disease as microalbuminuria is now seen as a sign of generalised endothelial dysfunction and large prospective studies have shown a strong correlation between glycemic control and microvascular complications[9]. Our chief role is therefore to detect these complications at an earlier stage to avoid the progression to irreversible stage.

So we need a cost effective and benefit method to identify those type 2 diabetic patients who need care and attention. Total leucocyte count is such a cost effective and easily available laboratory investigation that shows the overall inflammatory activity occurring in the body. Therefore we carried out a cross sectional study to correlate the relation between total leucocyte count, a biomarker of inflammation, and microalbuminuria a sign of endothelial dysfunction with cardiovascular risk factors among type 2 diabetes mellitus individuals for a better prognosis.

AIMS AND OBJECTIVES

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1. To find the correlation between total leucocyte count and microalbuminuria.
2. To find the correlation between differential count and microalbuminuria.
3. To find the association between total leucocyte count and cardiovascular risk.
4. To evaluate the relationship between total leucocyte count, differential count, microalbuminuria and cardiovascular risk factors.
5. To evaluate the other risk factors of diabetes like increased BMI, waist circumference, family history, and smoking with microalbuminuria, leucocyte count and cardiovascular risk.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORY OF DIABETES

In the 2nd century A.D, Roman physician Aretaeus used diabetes which means "to pass through". In 400-500 B.C, charaka and sushruta differentiated diabetes mellitus from diabetes inspidus. They recognised two types of diabetes, lean diabetes (**type 1**) and obese diabetes (**type 2**). They noted that diabetes runs in families and described polyuria and glycosuria (**honeylike urine).**[10]

In 1869, **Paul Lagerhans** from Germany discovered two systems of cells present in the pancreas. One set of cells secrete normal pancreatic juice, and the other set of cells are identified as 'Islets of Langerhans '. In 1901, Eugene Opie mentioned 'Diabetes mellitus' is due to Islets of Langerhans destruction partly or totally.

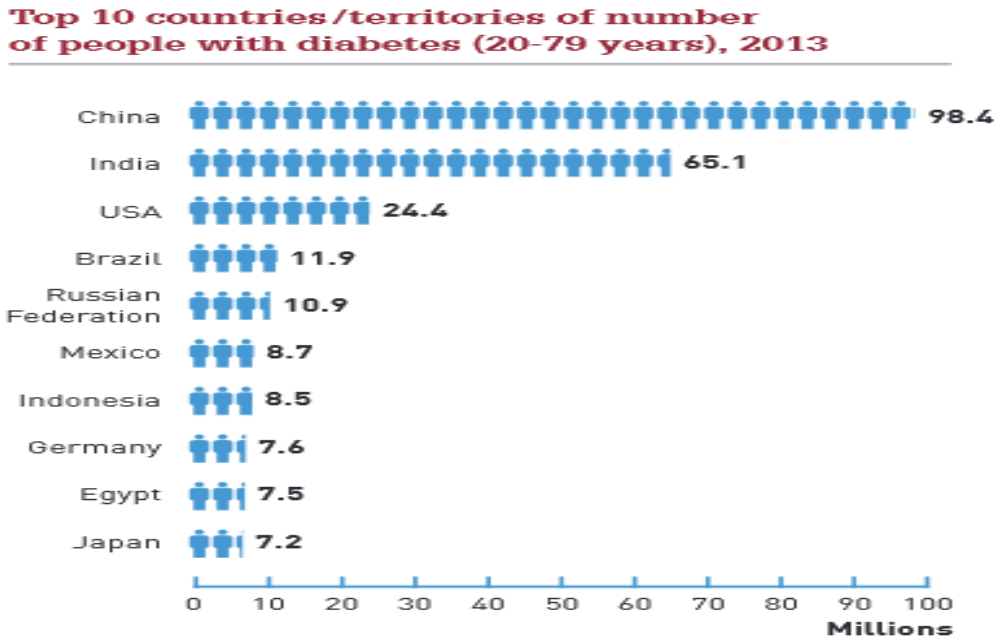
In 1920 **Frederick G. Banting**, an orthopaedic surgeon employed as a lecturer in physiology while preparing for the lecture thought a way to extract a substance from pancreas that can be used to reduce blood sugar level. He and his assistant a medical student **Charles H. Best** induced diabetes in dogs by removing the pancreas and tried to see whether glycosuria could be suppressed by administration of fluid isolated from Islet of Langerhans of other healthy dogs. In July 1921, first positive result came. Dog Marjorie was kept alive with pancreatic extract injections for 70 days. The extract named Isletin was

extracted in pure form with a technical guidance of Macleod and the help of expert biochemist J.B. Collip. In 1923 Nobel Prize in medicine was awarded to **Banting and Macleod**[11].

EPIDEMIOLOGY OF DIABETES MELLITUS

Diabetes mellitus is the most common metabolic disorder in the world. According to **IDF 382 million** people in the world live with diabetes as of **2013**. The number of individuals with diabetes is increasing steadily in most countries.

China has the largest number of diabetic individuals in the world (92 million), followed by India with 65.2 million[12]



In the year 1971 at the time of first national survey the prevalence rate was 2.3%in the urban areas and 1.2% in the rural areas. Now the prevalence was 15-20%in the urban areas and half of that in rural areas. India is currently

in the grip of an explosive epidemic of type 2 diabetes. As per the latest statistics, we have more than 60 million people living with diabetes in India and it is even worrying that more than 50% of individuals with diabetes remain unaware that they have this condition.

Type 2 diabetes mellitus comprises 85-95% of all cases of diabetes, type 1 diabetes contributes around 5% and other forms of diabetes are rare. There is an explosive increase in prevalence of type 2 diabetes in developing countries and steady increase in prevalence of type 2 diabetes in developed countries. The explosive increase in the prevalence of diabetes is due to the modernisation, urbanisation, and industrialisation leading to overweight and obesity in a genetically predisposed individual.

Type 2 diabetes is a disease of late middle age and occurs a decade earlier in India than in west and even in non-obese individuals. Self reported is 39.1%, undiagnosed is 60.9%, 25% of individuals have never heard of diabetes, 60% do not know that diabetes can lead to organ complications, more than 80% do not know the risk factors for diabetes, 60% do not know that diabetes can be prevented and 50% have poor glycemic control[13].

MORE THAN 50% OF DIABETIC SUBJECTS ARE UNDIAGNOSED

TYPE 2 DIABETES MELLITUS

NORMAL GLUCOSE METABOLISM

Dietary carbohydrates are the main source of energy. Major carbohydrates in diet are starch, glycogen, sucrose, lactose, fructose and cellulose. Complex carbohydrates are broken down into monosaccharides and then absorbed. The metabolic fate of glucose is determined whether the individual is in fasting or in the fed state. In the fed stage various biochemical processes are attuned towards energy storage. In the fasted state the process are directed towards breaking the energy depots and ensuring constant supply of glucose to the brain and of other metabolic fuels to the other tissues. The main storage form of glucose in animals is glycogen. Glycogen is a complex carbohydrate consisting of repeated units of glucose and found chiefly in the liver and skeletal muscles.

In the skeletal muscle it occurs as a readily available source of energy during muscle contraction, whereas in liver it contributes to the hepatic glucose output during fasting. Large amounts of lactate are generated during muscle contraction by means of anaerobic glycolysis. This lactate thus generated is transported to liver where it is reconverted to pyruvate and then to glucose. This process is a gluconeogenic mechanism and the blood sugar is kept constant even during strenuous physical activity. The lactate cycle is known as **Cori's cycle**.

Other substrates for gluconeogenesis include the glucogenic amino acid alanine and the glycerol part of neutral fat. In addition to liver, kidney is another major site for gluconeogenesis. However liver is the only organ where free glucose can be released into systemic circulation.

HORMONAL CONTROL OF GLUCOSE

For optimal functioning of all organs especially the brain, it is essential to maintain the blood glucose within a normal range. This requires the coordinated actions of various hormones acting together. Insulin is the only hormone which brings down the blood glucose level. Arrayed against it are a group of molecules commonly termed "counter- regulatory hormones".

Insulin Decrease Blood Glucose

By Promoting	By Inhibiting
Glycogenesis Lipogenesis Protein synthesis	Gluconeogenesis Glycogenolysis Protein breakdown Lipolysis

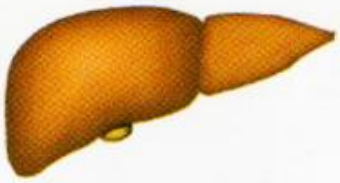
TYPE 2 DIABETES: MAJOR METABOLIC DEFECTS

Peripheral insulin resistance in muscle and fat

↑ Lipolysis
↓ clearance of triglyceride
↓ glucose uptake
↓ glucose utilization

↓clearance of triglyceride
↓ glucose uptake
↓ glucose utilization

Hepatic insulin resistance



↑VLDL production
↑ glucose output
↓ glucose uptake
↓ glucose utilization

Relative insulin deficiency

Insulin secretion not sufficient to overcome insulin resistance

Increased blood glucose (counter regulatory hormones)

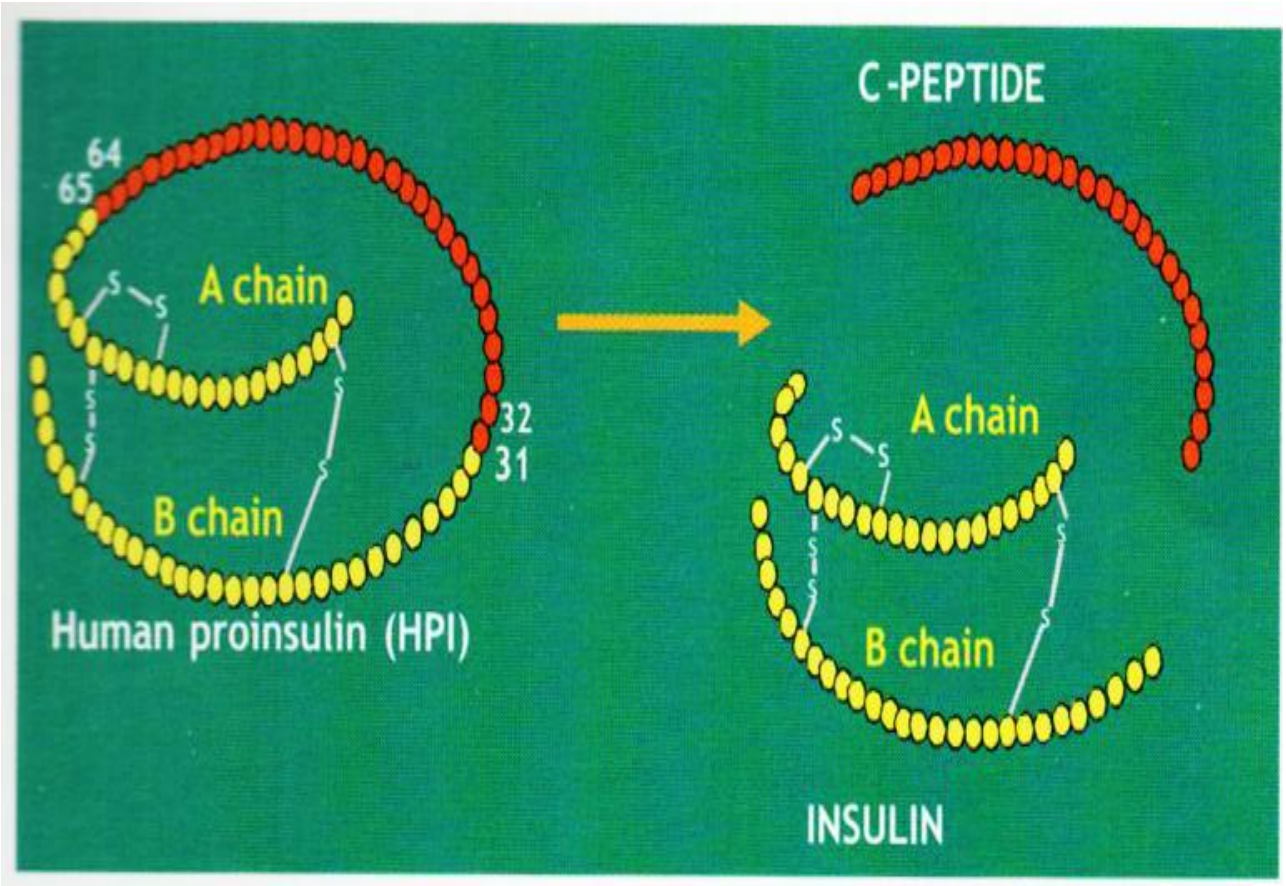
- **Glucagon** promotes glycogenolysis
- **Glucocorticoids** promotes gluconeogenesis
- **Growth hormone and catecholamines** promote glycogenolysis and gluconeogenesis.

In the fed state insulin promotes the glucose utilising and energy storing process (glycogen synthesis, fat synthesis (triglyceride), protein synthesis) and inhibits glucose producing process (glycogenolysis and gluconeogenesis) and lipolysis. In the fast state, the blood sugar levels are maintained by combination of inhibition of insulin release and increased action of counter -regulatory hormones.[14]

INSULIN

Insulin a polypeptide hormone is secreted by the beta cells of islets of langerhans of pancreas. Insulin is synthesised from its precursor preproinsulin, which gets broken down to proinsulin which in turn is cleaved to form equimolar concentration of insulin and C-peptide or connecting peptide. Insulin is thus stored in secretory vesicles in the cytoplasm of beta cells.

STRUCTURE OF INSULIN



When the individual is replete with glucose, e.g. after a meal, glucose enters the beta cell through glucose transporter-2 (**GLUT-2**), an insulin independent transporter. Adenosine tri phosphate is formed to yield energy when glucose is metabolised by the enzyme glucokinase.

High level of ATP in the cell lead to closure of a special transporter on the cell membrane called K-ATP(ATP sensitive potassium channel), leading to accumulation of potassium ions in the cytoplasm and depolarisation of the cell. This opens the calcium channels, influx of calcium into the cell and release of insulin from the secretory vesicles into the extracellular fluid and hence into the blood.

The glucose sensor of the beta cell is the enzyme glucokinase. Inactivating mutations of this enzyme lead to failure of the beta cell to secrete insulin even in the face of elevated blood glucose concentrations. Maturity onset diabetes of young (**MODY 2**), a monogenic form of diabetes is the heterozygous state of this mutation. Conversely, activating the mutations leads to hypoglycemia.

In addition to glucose, insulin secretion can be stimulated by certain amino acid particularly leucine, glucagon, incretin hormones (**GLP and GIP**) and parasympathetic stimulation. Insulin itself, sympathetic stimulation and certain drugs are inhibitors of insulin. In normal individuals the most potent stimulant for suppressing insulin secretion is fall in blood glucose levels.

Insulin secretion in response to blood glucose levels occurs in two distinct phases. The first phase is short lived, lasting for less than 5 minutes, which is produced by the release of insulin from preformed secretory granules. This **first phase** is important in suppressing gluconeogenesis and in tackling postprandial hyperglycemia, followed by second phase, which lasts for 30-60 minutes, depending on the nature of glucose load.

This **second phase** is produced by fresh synthesis of insulin and secretory granules are recruited from the storage pool. Type 2 diabetes is due to defect in the first phase of insulin secretion[15].

Insulin exerts its action on target cell by binding to insulin receptors on the cell membrane. The insulin receptors consist of alpha and beta subunits. The alpha subunits projects outside the cell surface and contains insulin binding sites. The beta subunit is partly intracellular and can act as a tyrosine kinase when insulin binds to the A subunit. This leads to auto phosphorylation of the receptor as well as a number of intracellular proteins, which include insulin receptor substrate (**IRS**) 1 and 2.

The second messengers like phospholipids are activated by phosphorylated proteins which mediate the various actions of insulin. The two major intracellular pathways of insulin are the phosphotidyl 3-kinase (PI3K) which mediates the metabolic pathway and the adapter protein (Grb-2-SOS) which mediate mitogenic pathway such as weight gain.

The most important action of insulin is the synthesis and translocation of glucose transporter 4(**GLUT-4**) to the cell membrane in adipose tissue and skeletal muscle, which is insulin dependent. Glucose uptake in other cells is insulin independent and depends upon the concentration of blood glucose.

Other actions of insulin are promotion of glycogen synthesis, protein synthesis, lipogenesis and inhibition of glycogenolysis, gluconeogenesis, proteolysis and lipolysis.

COUNTER -REGULATORY HORMONES

The chief effect of these hormones is to antagonize the biochemical actions of insulin. The important counter-regulatory hormones are

- Glucagon, from alpha cells of islets of langerhans
- Cortisol, from adrenal cortex
- Catecholamines (particularly epinephrine from adrenal medulla and sympathetic nerve endings).
- The action of glucagon and epinephrine are quick and short lived. On the other hand action of cortisol and growth hormone are slow to act, but long lasting. Glucagon acts mainly on liver to stimulate glycogenolysis and cortisol acts mainly on peripheral tissues to promote gluconeogenesis. In type 2 diabetes, in addition to insulin action deficiency, glucagon excess is also noted.

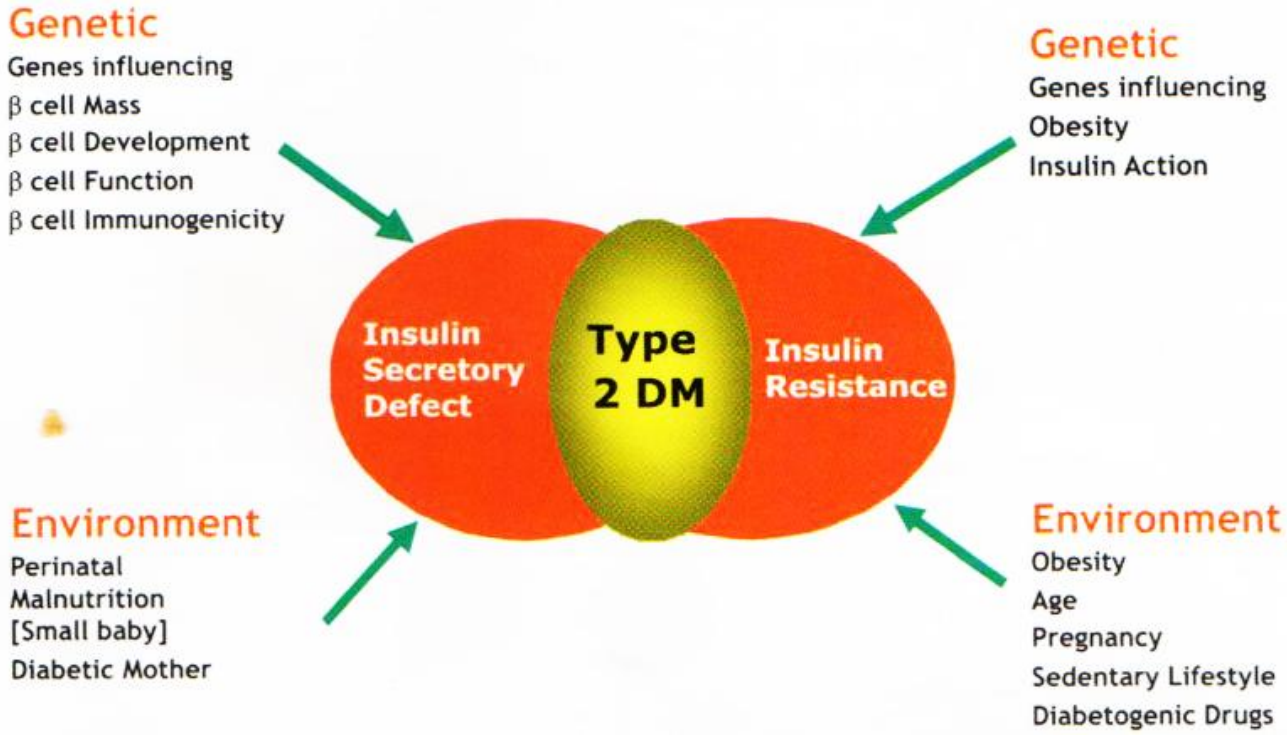
PATHOPHYSIOLOGY OF TYPE 2 DIABETES MELLITUS

The most common form of diabetes is type 2 diabetes and constitutes more than 90%. The etiopathogenesis of type 2 diabetes is complex and unraveled. The current understanding of the pathophysiology of diabetes according to latest American diabetes association represents a continuum of clinical scenarios , ranging from severe insulin resistance (IR) with relative insulin deficiency to severe insulin deficiency with some degree of insulin resistance. It is not clear which comes first, insulin resistance or insulin secretory defect.

All individuals become insulin resistance at some part of time. However, most of them have sufficient beta-cell reserve to overcome this IR and prevent the development of type 2 diabetes and individuals who develop type 2 DM might have a decreased beta–cell reserve, which renders them incapable of overcoming insulin resistance over a period of time. Insulin resistance can be acquired or inherited.

Most important of IR is obesity, particularly abdominal obesity which again can be due to genetic or environmental factors. Certain genetic syndromes, use of drugs like corticosteroids are associated with diabetes. Low birth weight predispose to the development of diabetes. Reversible causes of insulin resistance include physical inactivity and chronic hyperglycemia (glucotoxicity).[16]

GENES - ENVIRONMENT INTERACTION IN DIABETES

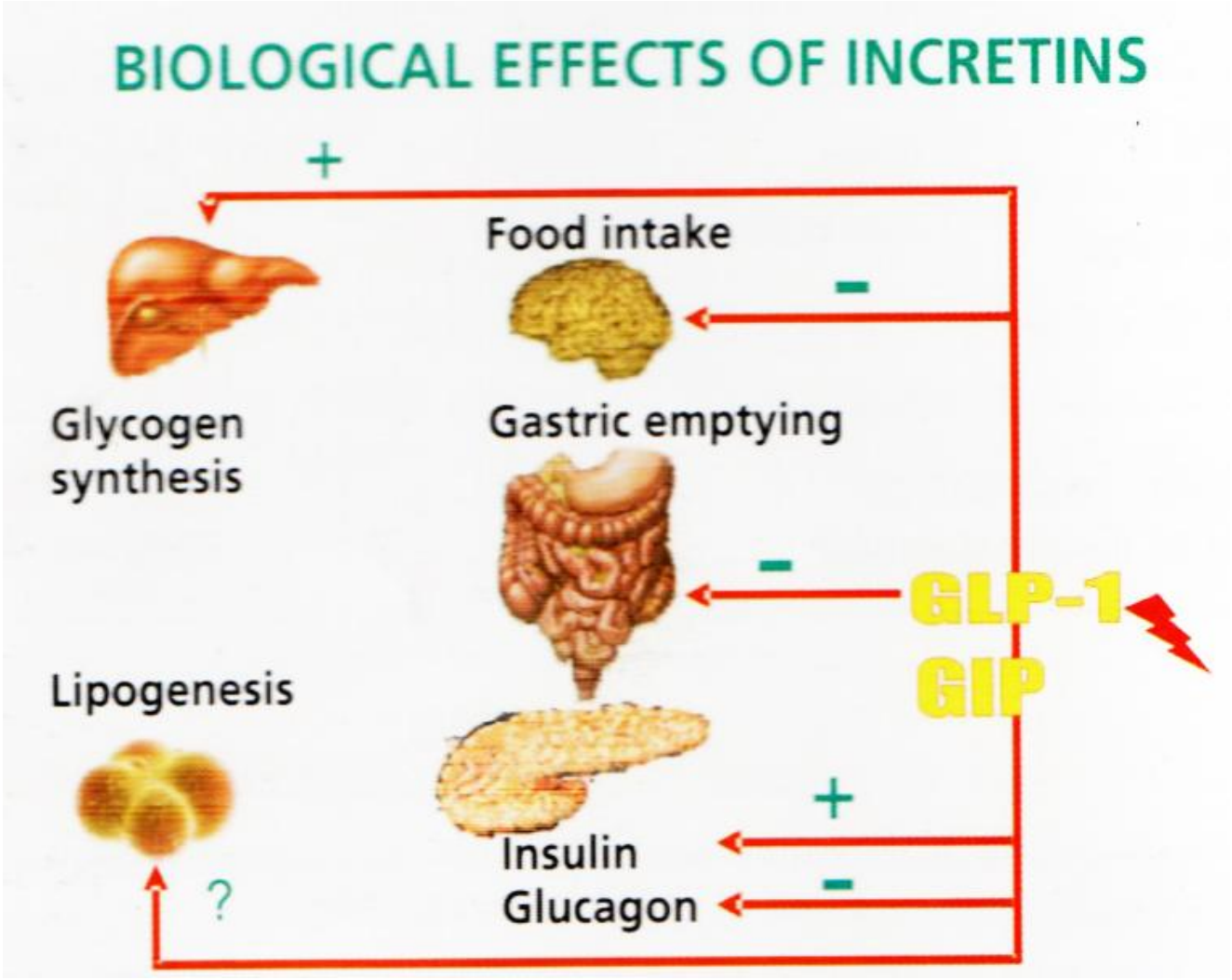


Resistance to insulin action can occur at the level of the insulin receptor or in the post receptor pathways. Down regulation of the receptors as well as anti-insulin and insulin receptor antibodies have been implicated in the etiopathogenesis of type 2 DM. It is of interest that resistance seems to develop only to the metabolic effects and not to the mitogenic effects.

The major contributor to beta cell dysfunction is genetic. The genes responsible for this impairment of beta cell function remain unknown. Even before the development of diabetes, beta cell dysfunction can be detected at the stage of impaired glucose tolerance as the beta cell defect is progressive. An average individual develops diabetes only after losing 50% of beta cell mass.

Environmental factors that contribute to beta cell dysfunction include chronic hyperglycemia, free fatty acids, alterations in incretin axis and in utero malnutrition. Functional defect rather than structural defect seems to be responsible for the beta cell dysfunction in type 2 DM.

In addition to insulin, two other hormone systems deserve special mention. The physiological antagonist of insulin is **glucagon**, which is secreted by alpha cells of pancreas in response to a variety of stimuli important one is decrease in blood glucose. It raises the blood glucose mainly by hepatic glycogenolysis and to some extent gluconeogenesis. In normal individuals, high blood sugar level leads to suppression of glucagon secretion. In type 2 diabetes, this feedback inhibition does not occur leading to inappropriately high glucagon levels, contributing to hyperglycemia.


BIOLOGICAL EFFECTS OF INCRETINS
+
Food intake
-
Glycogen synthesis
Gastric emptying
-
GLP-1
GIP
Lipogenesis
+
Insulin
-
Glucagon
?

The second group of hormones **"incretins"**. These are hormones secreted from intestinal epithelium in response to carbohydrate rich meal. They act on the pancreas and stimulate insulin release in response to blood glucose level. These are responsible for incretin effect and oral carbohydrate intake causes more insulin to release than intravenous glucose[17].

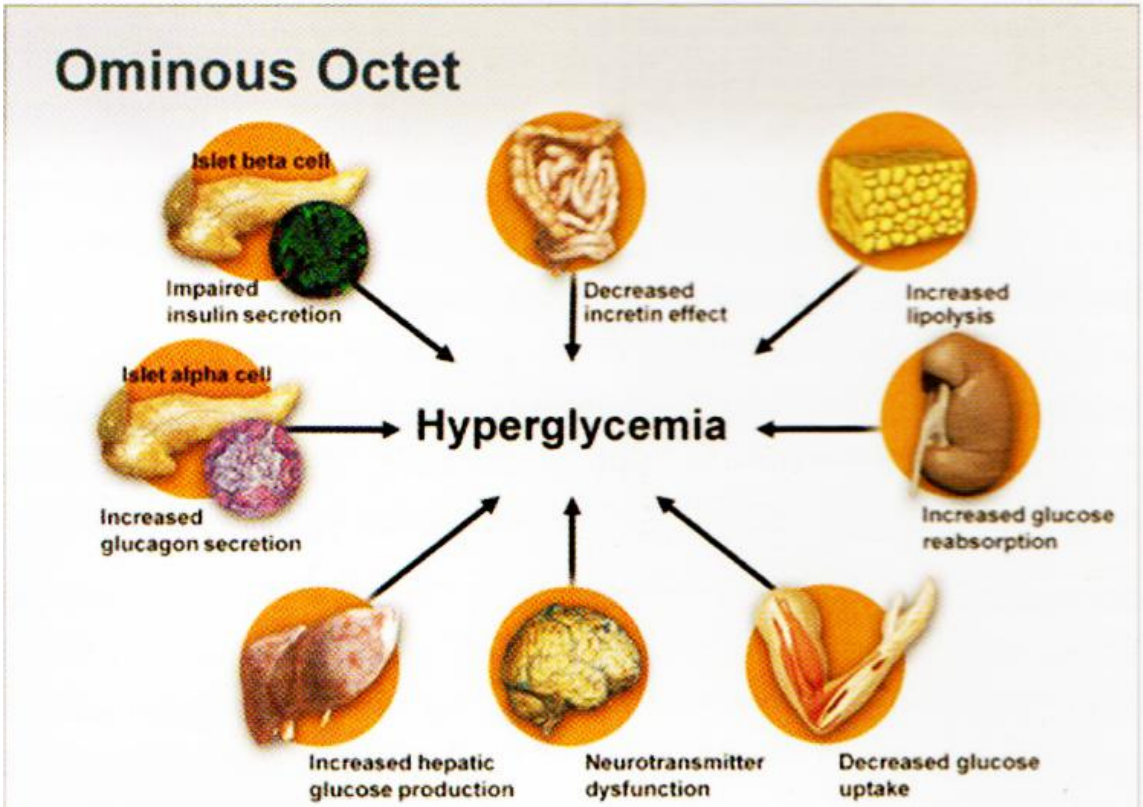
They also inhibit glucagon release from the alpha cell, slow gastric emptying and produce satiety and act on the central nervous system to reduce appetite and produce weight loss. The two important hormones in this incretin group are glucagon like peptide-**1(GLP-1)** and glucose dependent insulinotrophic peptide (**GIP**).These peptide have a shorter half life, since they are degraded by the enzyme dipeptidyl peptidase-4(DPP-4).

In type 2 DM, there is a reduction in the levels of both GLP-1 and GIP; whereas the response to GLP-1 is intact, that of GIP is diminished or absent in type 2 DM. In addition to the pathogenesis mentioned above, the role of other organs in the pathogenesis of type 2 DM has been termed as '**ominous octet'.**[18]

METABOLIC ABNORMALITIES AND HYPERGLYCEMIA IN TYPE 2 DM

Liver, skeletal muscle and adipose tissue are the major sites of insulin action. In short insulin promotes utilization of glucose, energy storage as triglycerides in adipose tissue and as glycogen in the liver. When IR develops,

ACANTHOSIS NIGRICANS



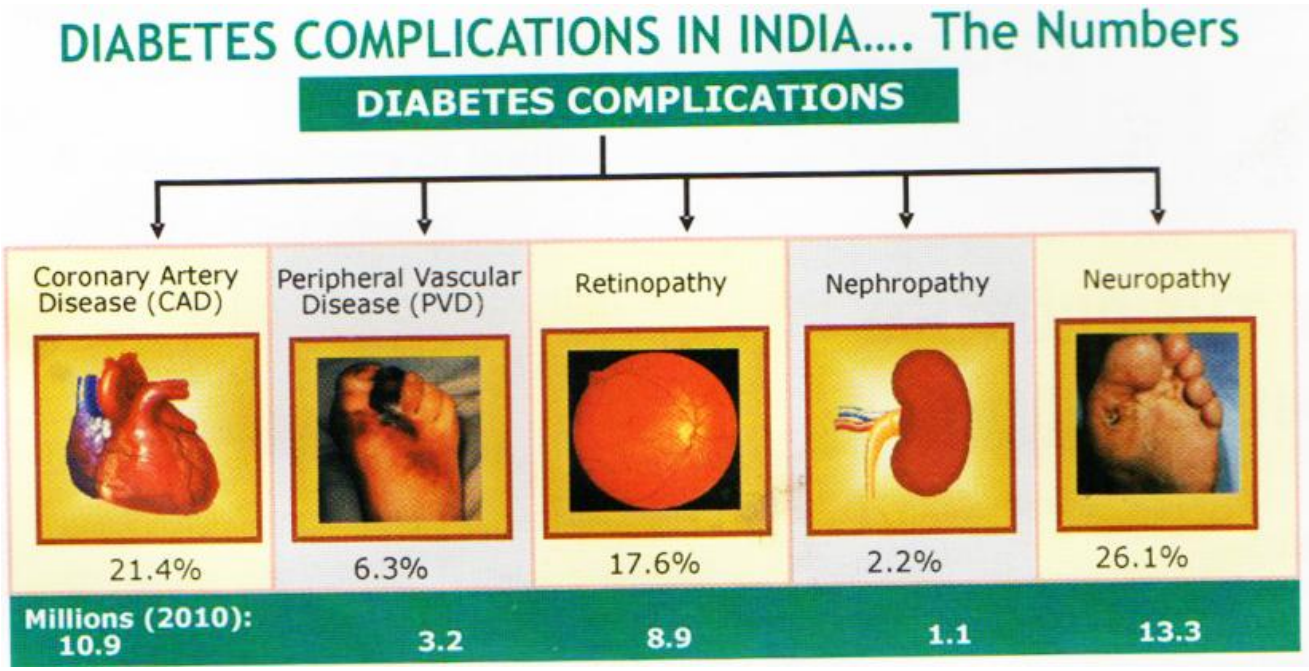
these processes either do not occur at the appropriate rates or occur only at the expense of raised insulin levels. In the process of developing Type 2 DM compensatory hyperinsulinemia is the earliest biochemically detected abnormality.

Acanthosis nigricans, a hyperpigmented velvety lesion of the skin, found on the posterior aspect of the neck, axillae, and groin is an important cutaneous marker and it is most often met with obese individuals with Type 2 DM or prediabetes. Epidermal cell growth by insulin or insulin like growth factor (IGF) is postulated to the development of acanthosis nigricans and is often associated with skin tags.

Inappropriate release of glucose due to defective insulin action in the liver leads to fasting hyperglycemia. Due to the action of insulin hepatic glucose output is completely suppressed after a meal. This suppression does not occur with insulin resistance, leading to postprandial hyperglycemia.

The main acute complications are diabetic keto acidosis and non ketotic hyperosmolar coma. The major chronic complications of diabetes are coronary artery disease **(CAD**) 21.4%, Peripheral vascular disease **(PVD**) 6.3%, **retinopathy** 17.6%, **nephropathy** 2.2**%, neuropathy** 26.1%.[19]

DIABETES COMPLICATIONS IN INDIA.... The Numbers



COMPLICATIONS OF TYPE 2 DIABETES MELLITUS

Microvascular complications	**Macrovascular complications**	**Other complications**
(1) **Eye Diseases**	(1**) Coronary Heart Disease**	(1**) Gastrointestinal Malfunction** (gastroparesis, diarrhoea)
Retinopathy (nonproliferative/proliferative)	(2) **Peripheral Arterial Disease**	(2**) Genitourinary Dysfunction** (uropathy/sexual dysfunction)
Macular edema	**(3) Cerebrovascular Disease**	**(3)Dermatological Problems**
(2)Neuropathy		(4 **Infections**
Sensory and motor (mono- and polyneuropathy)		(5) **Cataracts**
Autonomic neuropathy		(6**)Glaucoma**
(3) **Diabetic Nephropathy**		(7**)Periodontal disease**
		(8)**Hearing loss**

MICROALBUMINURIA

The major excretory organ in the body is the kidney. Toxic molecules, metabolic wastes (urea and creatinine) are filtered from blood by the nephron, the functional unit of the kidney and extra volume of fluid is also removed to maintain normal blood volume. Electrolytes like sodium, potassium and proteins are reabsorbed.

Each kidney consists of one million nephrons and each nephron contains glomerulus and tubules. Filtering membrane of the glomerulus consists of three layers; the endothelium, the epithelial podocyte, and the basement membrane.

The filtrate passes through the glomerular filtering membrane to reach the four parts of the tubular system: the proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting duct, where most of the reabsorption occurs.

Most of the glucose and plasma proteins gets reabsorbed in the proximal tubule ,concentration of urine occurs in the loop of Henle and sodium, potassium ions get regulated in the distal tubule[20].

The Blood supply to kidney is through renal arteries which branches into segmental arteries, then into different lobular arteries, again into interlobular arteries and finally divide to form afferent and efferent arterioles.

Afferent arterioles receive the blood, while efferent arterioles remove the blood, so both afferent and efferent arterioles play a key role in maintaining the glomerular pressure by vasoconstriction and vasodilatation.

Transportation of the important essential molecules such as hormones, lipids, vitamins, hormones, minerals and exogenous substance such as drugs are transported by the proteins. The oncotic pressure is maintained by the proteins between plasma and interstitial space.

Albumin, the carrier protein, immunoglobulin and acute phase proteins are the proteins present in the blood. Albumin is the most abundant, highly soluble, single polypeptide containing 585 amino acid sequences accounting for 60% of serum proteins with a concentration of 3.4 – 5.4g/dl[21].

The main contribution of colloid oncotic pressure is by albumin and its levels are affected by factors, insulin and cortisol. In the proximal convoluted tubule albumin and other proteins are reabsorbed by receptor mediated endocytosis. This endocytosis process is achieved by receptor complex called megalin-cubilin. Albumin gets attached to this complex in the apical membrane, an adapter at the tail of the receptor and finally internalisation of the ligand-receptor complex occurs.

After internalisation an endocytic vesicle transports the formed complex to the endosomal compartment and the protein complex is dissociated by acidification. In the lysosome, albumin gets degraded and the original aminoacids are returned to the blood stream.

Almost all the filtered albumin is reabsorbed by the kidney and only a small amount is excreted. **Normoalbuminuria** is defined when albumin excretion occurs at a rate of <30 mg/day; <20 microgram/minute in an overnight urine collection; <20mg/l on spot urine specimen or ACR <2.5mg/mmol in males or <3.5mg/mmol in females.

However in certain pathological conditions like diabetes, chronic renal disease, and hypertension, changes produced in glomerulus and tubules lead to the excretion of larger amounts of albumin leading to albuminuria. Albumin excretion can be expressed as a concentration of albumin in urine as mg/day or as albumin to creatinine ratio.

Microalbuminuria is defined when albumin excretion occurs at a rate of 30-300 mg/day; 20-200microgram/minute in a overnight urine collection ;20-200mg/L on spot urine specimen or ACR 2.5 to 25mg/mmol in males or 3.5 to 25 mg/mmol in females [22].

Macroalbuminuria or gross proteinuria is defined as levels exceeding 300mg/day or 200mg/l in spot urine or albumin-creatinine ratio (ACR) > 25 mg/mmol. This reflects a state of renal function deterioration. Protein excretion can be measured as total protein or specifically as albumin.

PATHOGENESIS

The exact pathology underlying the microalbuminuria in diabetes is not clearly understood. The main pathology of microalbuminuria in diabetes mellitus is endothelial dysfunction and insulin resistance. Hyperglycemia is the initiating factor in the pathogenesis of microalbuminuria. So microalbuminuria is reversible by improved glycemic control.

[23]Adverse effects of hyperglycemia is mainly diverse metabolic pathways due to

a) Advanced glycation end products,

b) Increased polyol pathway activation,

c) Protein kinase C activation ,

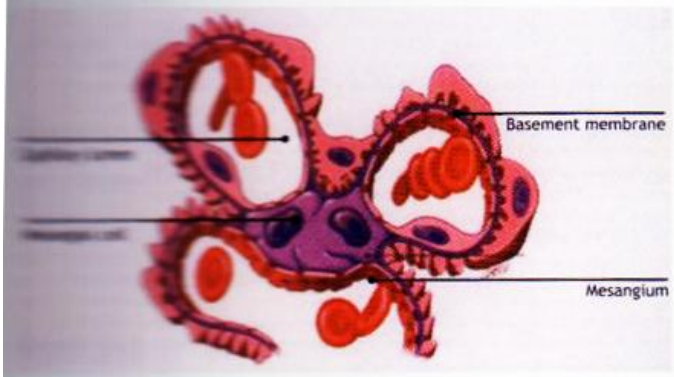
d) Increased flux through hexosamine pathway.

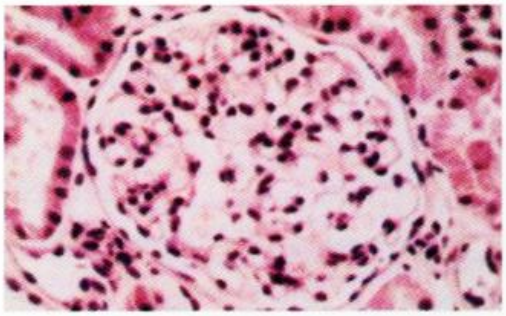
In addition to hyperglycemia, several other factors like increased blood pressure, morbid obesity, heavy exercise, various illnesses both acute and chronic and heart failure causes microalbuminuria.

Microalbuminuria occurs only after endothelial dysfunction. Degree of proteinuria poorly correlates with the podocyte injury. Indeed proteinuria can occur in the complete absence of structural changes of podocytes in type 2 diabetes.

Albumin excretion increases annually at a rate of about 20% in type 2 diabetes mellitus. Albumin excretion is determined by the amount filtered

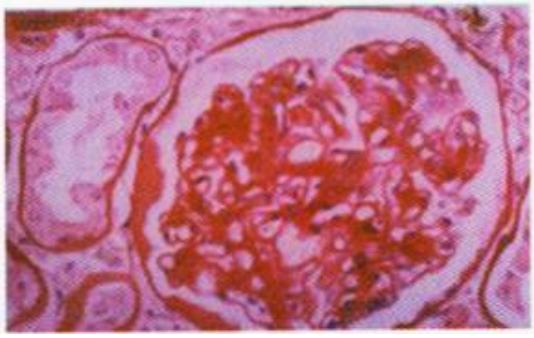
NORMAL GLOMERULUS

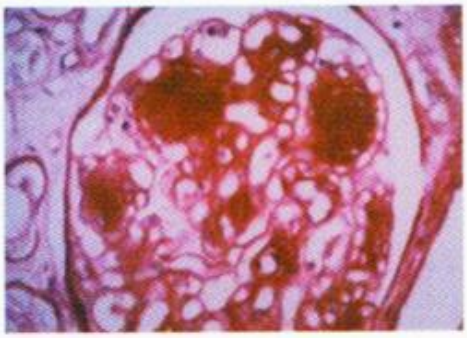




H & E Stain

DIABETIC GLOMERULOPATHY





across the glomerular capillary membrane and the amount reabsorbed by the tubular cells.

The passage of macromolecules across the glomerular capillaries is governed by the size and charge selective properties of glomerular capillary membrane and the hemodynamic forces operating across the capillary wall. Podocyte specific proteins in the regulation of the selective permeability have been recognised.

Until now, pathology of microalbuminuria in diabetes is related to insulin resistance or insulin resistance and obesity. Recently proinflammatory cytokines produced by visceral adipocytes (adipokines) is emerging as an important mediator of increased cardiovascular risk factor[24].

DETECTION

Microalbuminuria can now be detected by conventional dipstick method. These strips are inexpensive, easy to use but not accurate. Various other techniques are also available like immunoassays, and chromatographic techniques like high performance liquid chromatography. 24 hour urine collection is ideal and the results are accurate (Witte et al.), but complete compliance is not possible.

Overnight albumin excretion rate is simpler, easier way of timed microalbuminuria measurement (Dyer et al.,). The advantage of overnight urine collection is stable albumin excretion because of limited bed time movement.

Urinary albumin concentration (UAC) or Albumin creatinine ratio (ACR) on random samples (**American diabetes association,**) or first voided morning sample are more commonly used methods (**Lambers Heerspink et al)**. The easiest and convenient method for patients and practitioners is random spot collection. In this method the patients are instructed to collect the first voided urine after retiring from the bed and this method removes the biological variations, reflects the real magnitude of albumin excretion[25].

Timed urine collection			**Spot morning urine specimen**			
Albuminuria level	**24-hour albumin excretion (mg/day)**	**Overnight albumin excretion (µg/min)**	**UAC (mg/L)**	**ACR**		
				Gender	**mg/mmol**	**mg/ g**
Normal	<30	<20	<20	Male female	<2.5 <3.5	<20 <30
microalbuminuria	30-300	20-200	20-200	Male female	2.5-25 3.5-25	20-200 30-200
Gross proteinuria	>300	>200	>200	Male female	>25 >25	>200 >200

In Studies like **PREVEND** (Prevention of Renal and Vascular End stage Disease), researches compared ACR and UAC, both from first morning sample and random sample and the outcome was compared with 24-hour urine collection (**Witte et al.,**). First morning ACR sample correlated with 24-hour urinary albumin excretion (**UAE**).They are used in predicting morbidity and mortality associated with cardiovascular disease.

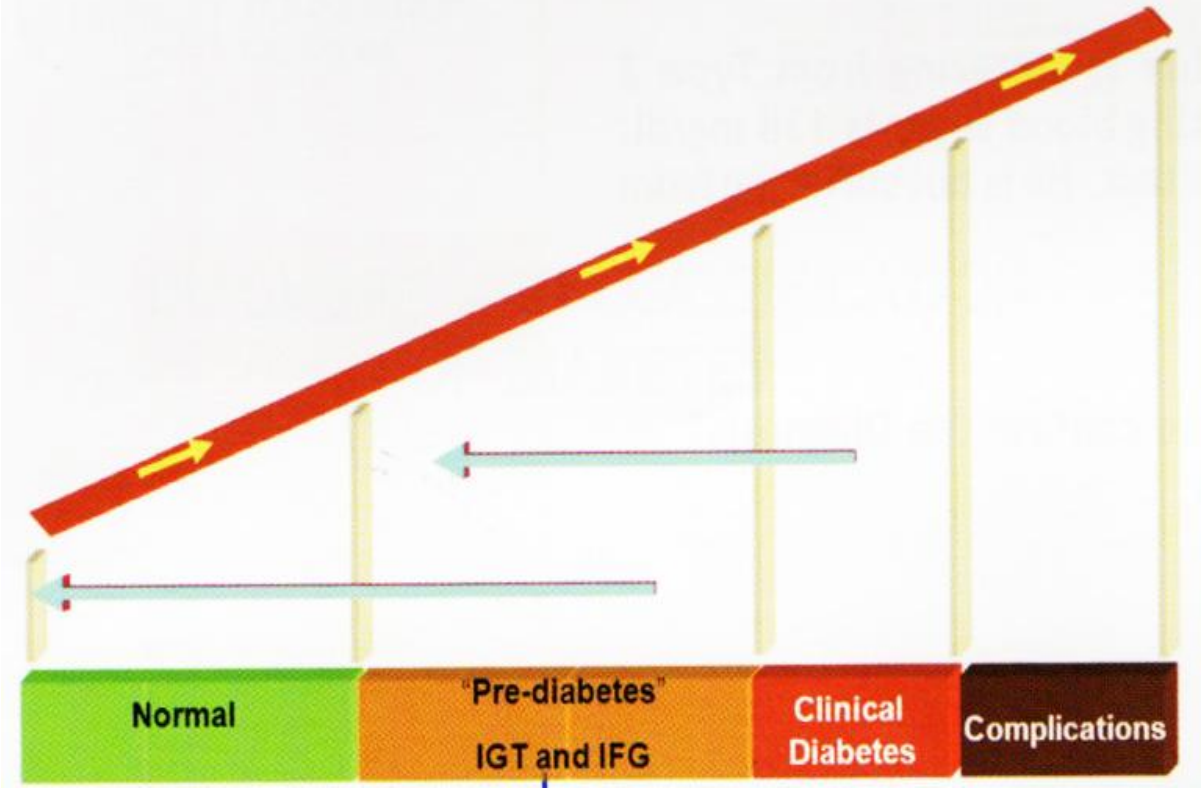
Several international guidelines recommend repeated samples for diagnosis of microalbuminuria (two of three samples must be positive) to remove the confounding factors (National Collaboration Centre for Chronic Conditions, 2008). But this seems difficult practically and single sample is used for majority of studies.

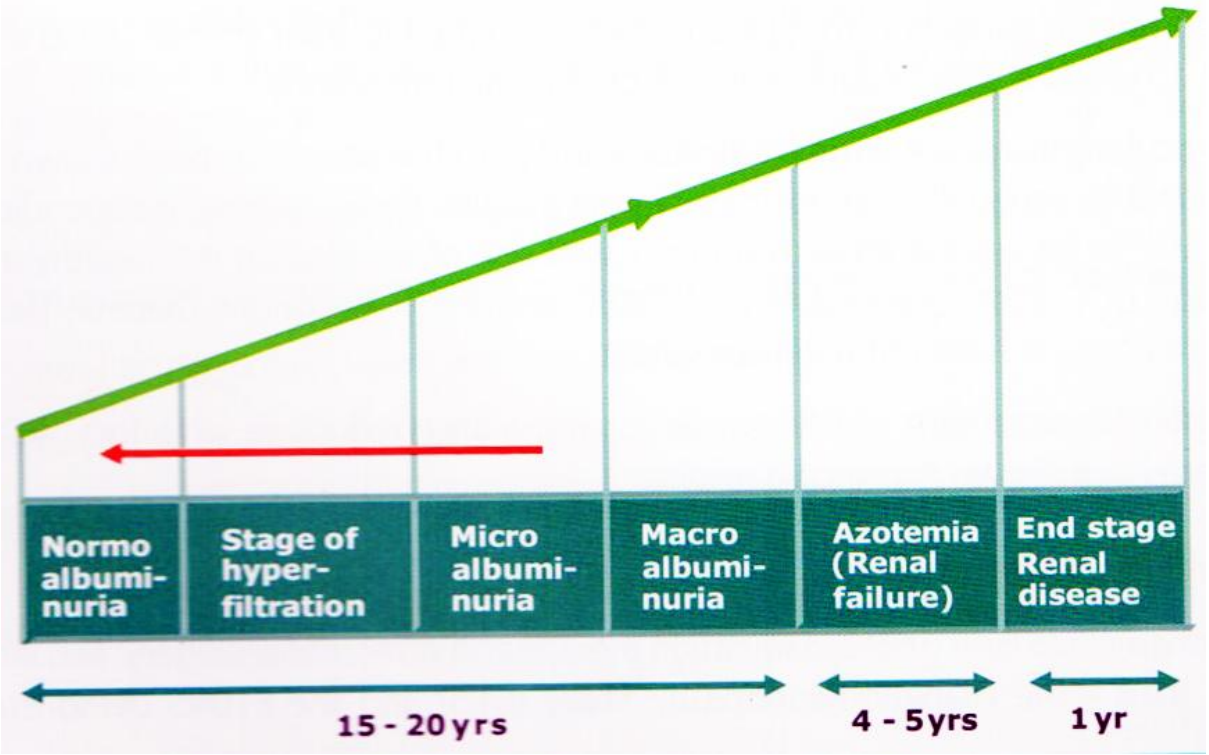
PREVALENCE OF MICROALBUMINURIA IN TYPE 2 DIABETES MELLITUS

American Diabetes Association, 2011 has recommended microalbuminuria screening for the management of diabetes in majority of the clinical guidelines. United Kingdom Prospective Diabetes Study (**UKPDS**) studied 5097 type2 diabetes patients from different ethnic groups for the prevalence of microalbuminuria and found it was 17% after 5 years, 25% after 10 years, and 28% after 15 years. In a population based study of type 2 diabetes **Vipputuri et al**[26], showed the prevalence of microalbuminuria and proteinuria were, 31%, and 12% respectively.

Prevalence of microalbuminuria varies according to the ethnicity and it is commonly found in Asians and black populations compared with whites (**Summerson et al)**. A comparative study between white Europeans and south Asian population revealed that microalbuminuria was more prevalent in south Asians (31%vs 20%) (**Dixon et al).** No difference between age, sex, and blood pressure was noted among individuals in this study.

NATURAL HISTORY OF DIABETES





Tillin et al[27] compared the prevalence of UAE among the Africans, south Asians, and white Europeans and showed that microalbuminuria was more prevalent among African people than south Asians and Europeans. In various studies done on diabetic patients attending diabetic clinic, showed the prevalence of microalbuminuria was higher among Asian population than white Europeans.

The relation between microalbuminuria and gender varies. **Pontremoli et al.,** 1997 have shown greater prevalence in males. Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications (**DCCT/EDIC)** have also shown that urinary albumin excretion in male gender is greater and it is associated with central obesity. EPIC-Norfolk study, the prevalence was higher among females (**Yuyun et al**). **Calvino et al,.** reported that there was no difference among either sex.

MICROALBUMINURIA AND RENAL FUNCTION

Deterioration of renal function is best recognised by microalbuminuria. **Levey et al,** reported that microalbuminuria is associated at an early stage of the kidney disease. **Astor et al,** recommended the use of albuminuria with eGFR for the prediction of End Stage Renal Disease (ESRD). **Behrane et al,** [28] 2011 reported recently reported the same. Therefore, high risk individuals should be routinely screened to identify microalbuminuria earlier to prevent or delay possible progression to ESRD.

MICROALBUMINURIA AND CARDIOVASCULAR RISK

Since microalbuminuria reflects the generalised endothelial dysfunction, it is now linked with cardiovascular abnormalities like CHD, myocardial infarction, heart failure, hyperlipidemia, and atherosclerosis. **Klausen et al.,** 2004 suggested the prognostic value of microalbuminuria for ischemic heart disease involving different population. After adjusting for known factors such as blood pressure, lipids, gender and BMI, the relative risk developing ischemic heart disease associated with microalbuminuria was 3.5.

The study by **Apostolovic et al[29],** documented that about one third patients with myocardial infarction had increased UAC and it was a strong predictor for myocardial infarction complications including mortality.

INFLAMMATION

The word inflammation meaning ` setting on fire ´ (16 century) is a local protective response to tissue injury. These processes have been known since Egyptian times 2500B.C. The cardinal signs of inflammation are rubor, calor, tumour, dolor was described by Celsus in first century A.D, and loss of function was later added by Galen 130-200 A.D. Vasodilatation, increased capillary permeability and interstitial fluid permeability, accumulation of leucocytes and stimulation of nerve endings by mediator such as substance P are the microscopic findings.

Ross et al[30] showed that cells such as monocytes, macrophages, T-cells, endothelial cells, release inflammatory mediators including cytokines and

chemokines during the process of plaque formation. This develops an inflammatory response in the vessel.

Adipose tissue secretes cytokines which is responsible for insulin resistance in liver, skeletal muscle, and vascular endothelial tissue and ultimately results in clinical expression of type 2 DM and cardiovascular risk

Endothelial production of adhesion molecules including E-selectin, intercellular adhesion molecules (**ICAM-1**), Vascular adhesion molecule (**VCAM-1)** are the crucial mediators of endothelial dysfunction in capillary and arterial endothelium are stimulants by TNF-alpha, IL-6 and CRP apart from promoting insulin resistance (**Gabay and Kushner ;Targher et al)[31].**

Tissues produce major proinflammatory cytokines like IL-6, activated leucocytes, adipocytes and endothelial cells. IL-6 induces gluconeogenesis, hyperglycemia, and compensatory hyperinsulinemia in rodents. Subcutaneous administration of recombinant IL-6 (**Tsigos et al**) produces similar results in humans.

Inflammation plays a major role in the etiology of type2DM and demonstrated an elevated level of IL-6 and CRP in type2 DM (**Sattar et al**).

Metabolic syndrome and type 2 DM are associated with markers of inflammation and levels of inflammatory markers were due to insulin resistance, but not due to insulin secretion.

A number of studies have associated routine hematological parameters with type 2 DM and cardiovascular disease and found that a strong association

exists between insulin resistance, cardiovascular disease and hematological parameters.

INFLAMMATORY MARKERS

Libby et al[32], mentioned that great work has been done to detect the inflammatory markers that are risk factors for coronary heart disease. Fibrinogen, C - reactive protein, total leucocyte count and Interleukin-6 are the most widely used markers and these are the acute phase reactants. Their presence in the blood reflects the current inflammatory state of the individual, with **leukocyte count and C-reactive protein** routinely used to measure the inflammation.

INFLAMMATION AND TYPE 2 DM

Diabetic individuals are at increased risk for cardiovascular disease because of atherosclerosis due to Advanced Glycation End products (**AGE).** AGE's are accumulated due to hyperglycemia and indicates metabolic burden, oxidative stress, and inflammation **(Xiong et al, Stern et al) .**[33]

AGE's and AGE specific receptors produces inflammatory reactions and endothelial dysfunction. AGE's produces unusual cross links in collagen leading to vascular stiffness.

Recent studies have shown the increased serum levels of AGE's in type2 DM patients with Coronary Heart Disease (**CHD**). AGE's accumulate in

coronary atherosclerotic plaques and cardiac tissue of type 2 DM in several immune histochemistry studies (**Nakamura et al, Grillo and Colombatto).**

Glucose reacts chemically and nonenzymatically with proteins in vivo to form amadori products. Amadori products are rearranged to form AGE's **(Letonja et al , Petrovic et al)[34].**

The significance of oxidising conditions and reactive oxygen species involved in the formation of glycol oxidation products which are a major class of AGE's that accumulate in diabetic tissues was studied by **Baynes and Thorpe el al,.**

Diabetic microvascular complications are capillary basement thickening and hypertrophy of extracellular matrix. Hyperglycemia leads to accumulation of AGE's in tissues causing tissue damage and diabetic complications **(Meerwaldt et al).[35]**

Excessive production of free radicals like reactive oxygen species, increased oxidation of substrates and auto oxidation of glucose leads to oxidation stress in diabetes. This free radical leads to reduction of nitric oxide, leading to vasoconstriction and endothelial dysfunction

INFLAMMATION AND LIPOPROTEINS

Earlier the complement complex C5b-9 gets activated in the atherosclerotic rich lipid core. Local cellular gene expression and behavior are influenced by cytokines. The production of collagen by smooth muscle is enhanced by Platelet Derived Growth Factor **(PDGF**), fibroblast growth factor, transforming Growth Factor –beta **(TGF-β**) and macrophage inflammatory responses were suppressed.

CD40 ligand on the T lymphocyte exerts a critical pro-inflammatory effect on atherosclerosis and gamma interferon (**IFN γ)** modulates the process of atherosclerosis. Endothelial anticoagulant- procoagulant balance is shifted towards procoagulant effect by inflammatory cytokines interleukin -1**(IL-1β**), and tumour necrosis factor –α (**TNF-α).**

NFKB family integrates various factors into inflammatory responses. Genes with NFKB response elements are vascular cell adhesion molecule-1, macrophage chemo tactic peptide -1, tissue factor are involved in proliferation of cell.

Atherosclerosis is a chronic form of inflammation present in the wall of the artery and epidemiological studies linked circulating inflammatory markers and cardiovascular events that were used in the prediction of cardiovascular risk taking into account other risk factors **(Hansson et al).**[36]

CARDIOVASCULAR RISK FACTORS

The main modifiable risk factors are[37]

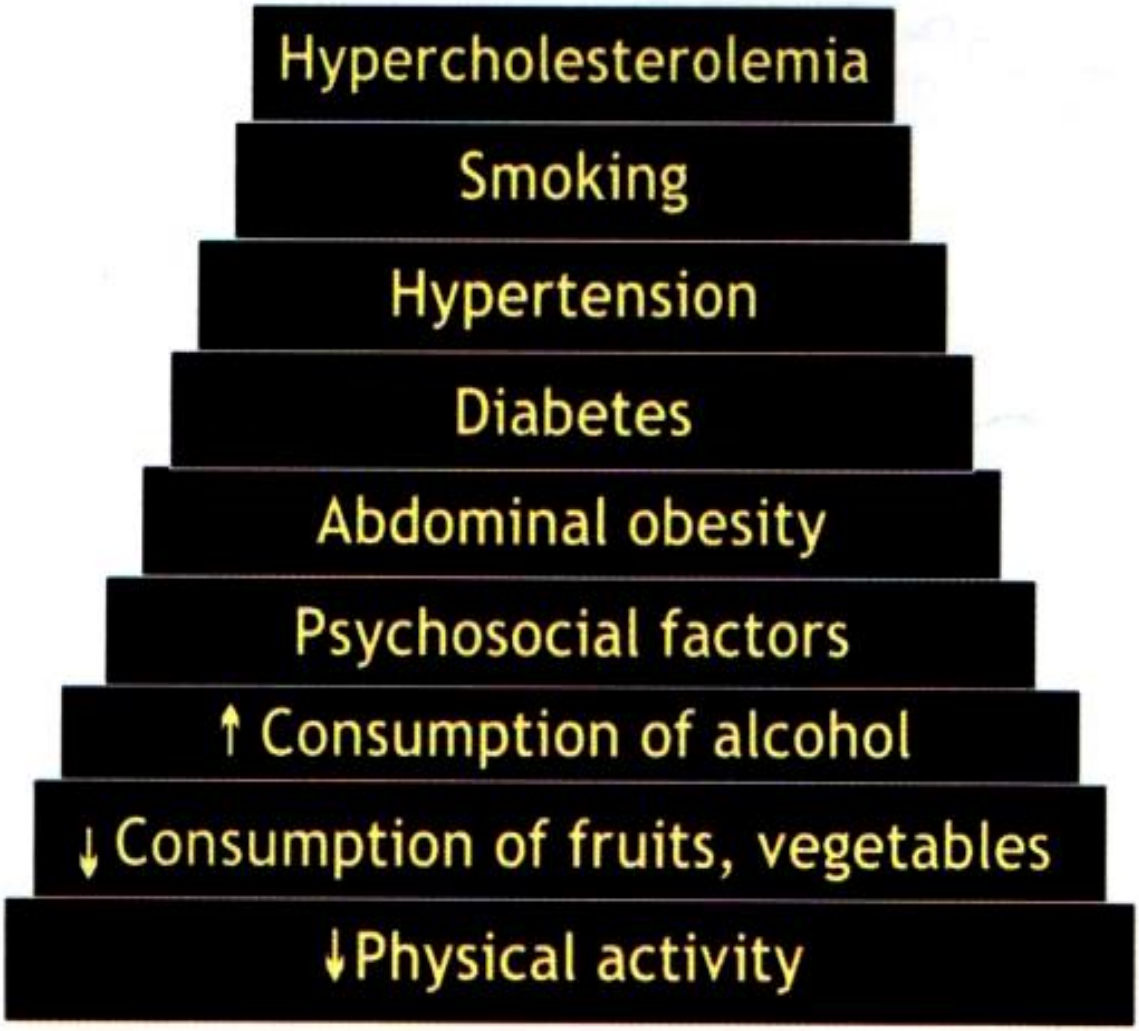
- Elevated blood cholesterol
- High triglycerides
- Elevated blood pressure
- Diabetes
- Smoking
- Obesity
- Sedentary life style

LIPOPROTEIN

Lipoprotein plays a key role in atherosclerosis and 60-75% of diabetic people die of myocardial infarction or stroke, therefore lipoprotein reduction can reduce the diabetic cardiovascular risk[38]. The stiffness and viscosity of lipid bilayer of membrane is increased when cholesterol / phospholipid ratio gets increased resulting in decreased permeability of cell membrane to water and other organic molecules.

Apart from lipoprotein abnormalities atherosclerosis, a serious complication of diabetes is due to the accumulation of advanced glycation end products in arterial tissue and numerous gene products try to restore the membrane homeostasis. So in atherosclerotic plaques these mechanism fail and vascular

RISK FACTORS FOR CVD



cells may die because of over accumulation of cholesterol which disrupts critical membrane function.

The fuel for skeletal muscle, heart muscle, and most other tissues is supplied by fatty acids. Esterification of these fatty acids forms triglycerides and cholesterol esters for transportation and storage which is water insoluable.

Over accumulation of cholesterol or fatty acids resulting in disruption of cell membrane structure or function is prevented by esterification reactions, representing cellular defense mechanism. Triglycerides and cholesterol esters are non toxic because of their minimal presence in cell membrane as oily droplets.

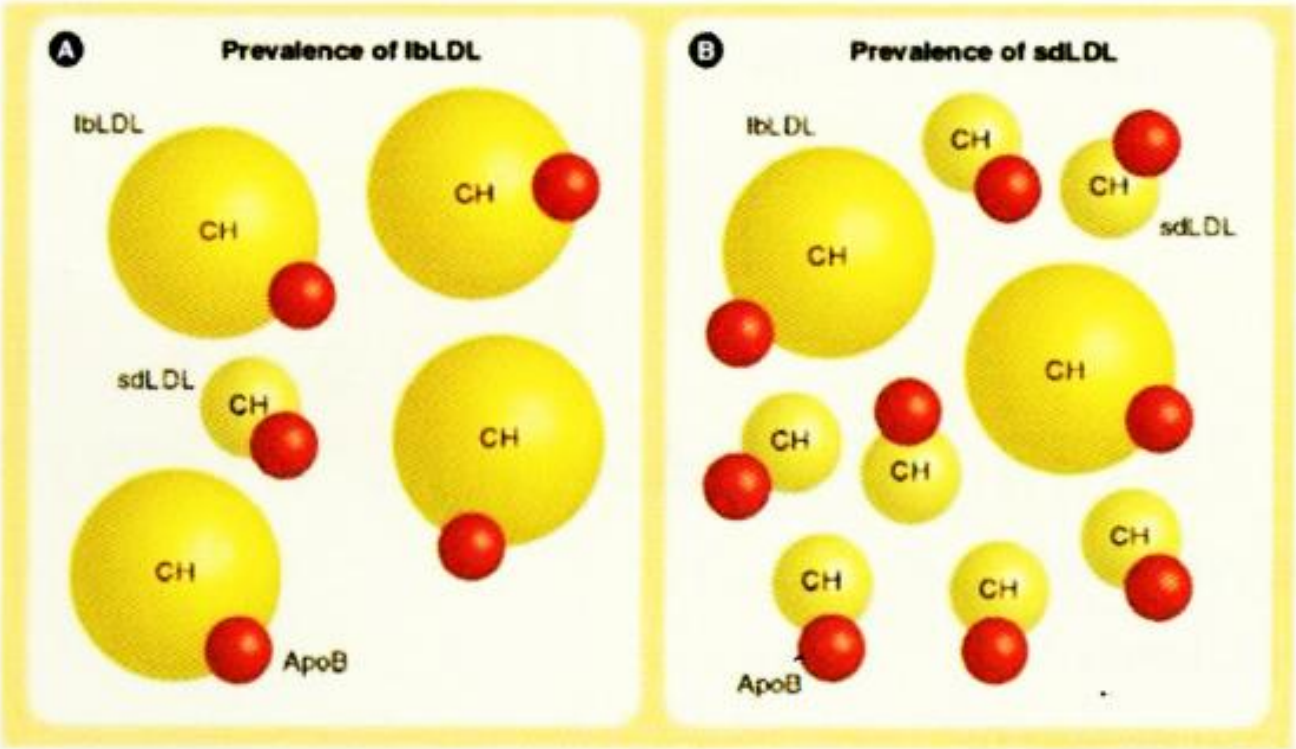
APOLIPOPROTEINS AND LIPOPROTEINS

The plasma lipoproteins are the pseudomicellar structure consisting of cholesterol, cholesterol esters, triglycerides, and phospholipids packed with specific proteins (apoproteins) for circulatory transport.

Based on the equilibrium densities by ultracentrifugation plasma lipoproteins are divided into chylomicrons, VLDL, HDL, and LDL. Chylomicrons, VLDL have abundant core lipid than surface phospholipid and HDL have more surface phospholipid compared to core lipid.

The lipoprotein structure and lipid transport are determined by the apoproteins. Apolipoprotein A-I and Apo-B are considered as basic structural apolipoproteins. The production and release of VLDL is stimulated, attributing

SMALL LDL



lbLDL – Large buoyant LDL

sdLDL - Small Density LDL

to hypertriglyceridemia leading to have low levels of high density lipoprotein (HDL) in their circulation. Also, higher levels of VLDL lead to increased exchange of cholesterol ester and triglyceride between VLDL and low density lipoprotein (LDL), increasing the triglyceride content of LDL and making them more susceptible to degradation by hepatic lipase, leading to formation of small dense LDL particles.

The lipid profile in type2 DM individual is characterised by high triglyceride levels, low HDL cholesterol levels, and normal levels of LDL cholesterol, with relative increase in the number of highly atherogenic small dense LDL particles.

In adipose tissue defective insulin action leads to excessive breakdown of triglycerides and formation of non–esterified fatty acids (NEFA). High levels of NEFA can stimulate gluconeogenesis in liver and can reduce insulin stimulated glucose uptake by tissues. Even though lipolysis occurs, ketone bodies are not produced in type 2 DM as insulin deficiency is not absolute and even a small amount of insulin is sufficient to prevent the formation of ketone bodies than to maintain glucose homeostasis.

Insulin has beneficial effect on platelet function, vascular integrity and the autonomic nervous system. Insulin resistance can adversely affect all these, causing increased susceptibility to ASCVD seen in type2 DM.

Lipoprotein (a) resembles low density lipoprotein and Lipoprotein (a) account for most of plasma “LDL” is associated with coronary and other atherosclerotic disease. In diabetes and insulin resistance Lipoprotein (a)

alterations occurs, but they play a minor role. Lipoprotein (a) measurement plays a key role to evaluate a cardiovascular risk due to genetic, and unmodifiable influence.[39]

LIPOPROTEINS AND ATHEROSCLEROSIS

The concentration of LDL in the arterial intima is abundant, stagnant and its concentration is equal to its concentration in blood plasma. In other tissues it is present to about one tenth of plasma. Collagen, elastin, proteoglycans of arterial intima are exposed to greater concentration of LDL.

Arterial intima is lacking the lymphatic vessels and so excess macromolecule species are not washed away. Enzymatic, Oxidative and other process disrupting the individual LDL particles are increased by greater residence time and high levels of LDL. Therefore the arterial intima was considered as a pool for LDL[40].

ACCUMULATION OF LIPID-LESION PROGRESSION

Earliest atherosclerotic lesions that appear in the coronaries and aorta of teenagers are the fatty streaks. These lesions serve as precursors for advanced lesions but never thicken the intima nor predispose to thrombosis. Histologically, macrophages filled with lipids and smooth muscle bearing lipids are present in the fatty streaks. Immune process, smoking, or hypertension cellular adhesion molecules are upregulated by

hypercholesterolemia, and macrophages from circulating monocytes move from blood into the fatty streaks.

Starting at the age of 20, **fibrous plaque** begins to appear from pre-existing fatty streaks. The intimal thickening is due to the proliferation of smooth muscle cells, macrophages, collagen, and fibrous tissue proteins

Once the plaque begins to grow and impinge on the arterial lumen, entire artery undergoes arterial enlargement which is depends on endothelial regulation of arterial lumen produced by velocity of blood and shear rate of wall.[41] Collagenous lesion extends around the circumference of the artery due to the failure of compensatory mechanisms and diabetes, leads to the failure of endothelial dependent relaxation of arteries resulting in increased atherogenic events.

Lipid rich core is the most dangerous part of atherosclerotic lesion. The fibrous cap is eroded by lipid accumulation and cellular apoptosis [42]. The blood is directed into the core when this cap ruptures.

PLAQUE STABILIZATION

Atherosclerotic intimal thickening is decreased by atherosclerotic regression resulting in reduced stenosis and this causes plaque stabilization.

Hypercholesterolemia interferes with normal endothelial function and decrease in LDL cholesterol normalizes relaxation produced by endothelial tissue improving the resistance produced by thrombosis also.

Lowering of lipid causes disappearance of macrophage foam cells from the lesions thereby decreasing the propensity to rupture leading to plaque stabilization and preventing other atherothrombotic events.

CHOLESTEROL

The absorption of 30-60% of cholesterol entering the intestine is unregulated. The daily biliary cholesterol is 900 mg and dietary cholesterol is only about 300-400 mg. Bile acids solubilise cholesterol in the intestinal lumen to form mixed micelle. Incorporation of the micelles is competitively inhibited by plant sterols and stanols which reduce plasma cholesterol.

Intestinal cholesterol asses into the absorptive cells by facilitated diffusion involving the recently discovered protein like Niemann pick. Cholesterol present in the cells undergoes esterification which is secreted into intestinal lymph. Out of this one third of cholesterol is added to total body stores from the diet and two thirds is synthesised.

REVERSE CHOLESTEROL TRANSPORT AND HDL

Most of the cholesterol in the body is made in the peripheral tissue cells. Excess cholesterol which cannot be degraded or metabolized by most cells is transported for disposal to liver. This is "**reverse cholesterol transport**" and mechanism of this is not clearly understood. Heredity, hormones, obesity and exercise all affect HDL2 primarily[43].

LDL AND HEPATIC CHOLESTEROL METABOLISM

Reverse cholesterol occurs in the liver and from here VLDL and LDL is delivered to body tissues. In the tissues VLDL is transformed into IDL and subsequently into LDL. LDL particles are taken up by receptor mediated endocytosis by peripheral tissues.

In liver about two thirds of total LDL catabolism occurs as it contains LDL receptors. LDL concentration in the plasma is often decreased by upregulating LDL catabolism and hepatic LDL receptor activity, than decreasing LDL production.

Hepatic cholesterol balance requires input and output. Bile acids undergo an enterohepatic circulation. The major route of cholesterol elimination is by faecal loss, bile acids, and their colonic bacterial degradation product.

Increased cholesterol excretion leads to the conversion of more amount of cholesterol to bile acids and more cholesterol is also synthesised in the liver. Ultimately cholesterol present in liver decreases, LDL receptors are increased and LDL level in plasma falls.

TRIGLYCERIDES

For intestinal absorption of fat, pancreatic lipase has to hydrolyse triglycerides to fatty acids and monoglycerides. In the intestinal lumen bile salts help in lipolysis and absorption of fatty acids by forming micelles. Inside the cells of intestine present in the mucosa, fatty acids are reesterified to

glycerol and arranged in chylomicrons in the triglyceride core. These chylomicrons enter the circulation via thoracic duct.

Chylomicrons carry triglycerides synthesised in the gut and VLDL carry triglycerides by the liver. Lipoprotein lipase metabolizes both these triglyceride rich lipoproteins in the peripheral circulation. Lipoprotein lipase enzyme hydrolyses only the triglycerides but not the cholesterol esters or fatty acids and so these are released into the circulation to enter into the endothelium of capillaries and parenchyma of tissue.

So triglyceride rich lipoprotein shrinks in size and hepatic cells take up the chylomicron remnants. In the hepatocytes VLDL is converted to LDL by the action of hepatic lipase.

Hypertriglyceridemia occurs when lipoprotein lipase is absent genetically. Severe deficiency in insulin can also decrease activity of lipoprotein lipase with the same result.

Insulin and plasma free fatty acids strongly influence the metabolism of hepatic free fatty acid and triglycerides. The liver takes the plasma free fatty acids and incorporates into triglycerides and forms VLDL.

Obesity is associated with high plasma triglyceride level because of high plasma free fatty acids. Deficiency in insulin, intake of alcohol excess also increases secretion of VLDL and plasma triglyceride levels are increased by increased plasma free fatty acid levels.

DIABETIC DYSLIPIDEMIA

The diabetic dyslipidemia is increased concentration of lipoproteins rich in triglycerides, decreased concentrations of HDL, LDL and HDL becomes a smaller, denser particles.

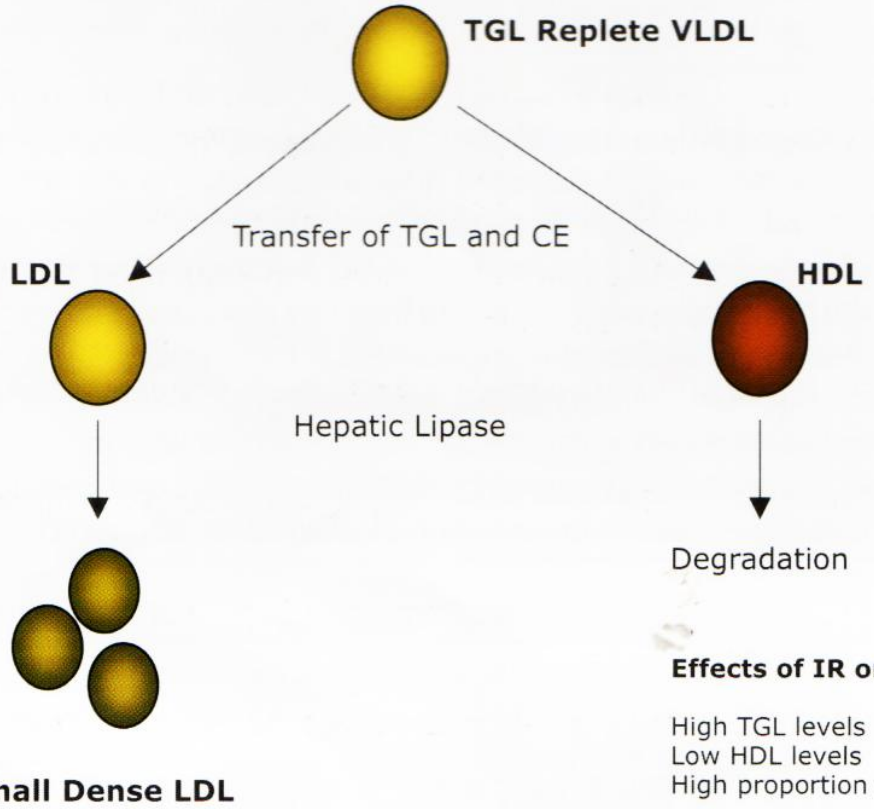
Increased movement of fatty acids to liver from adipose tissue is the primary abnormality in diabetic dyslipidemia. Hormone sensitive lipase in adipocytes is suppressed by insulin resistance, insulin deficiency or both, which causes release of NEFA into plasma.

Normally liver packs and exports the fatty acids as triglycerides in Very Low Density Lipoprotein, and VLDL levels are raised in plasma. By action of lipoprotein lipase the fatty acids from VLDL triglycerides are transported back to adipose tissue. However in insulin resistance or insulin deficiency state lipoprotein lipase activity is reduced and VLDL triglyceride level in plasma is raised.

Small LDL is formed as the result of major pathophysiological process. Per particle of Very Low Density Level in diabetic individuals or insulin resistant individuals have more triglycerides than VLDL in an average individual. Secondly, the exchange of neutral lipids in core- triglycerides and cholesterol ester in particles of lipoprotein is mediated by cholesterol ester transfer protein (CTEP).

When VLDL is abundant, then triglycerides replace cholesterol ester in both HDL and LDL. The hepatic lipase removes the lipid present in the core of triglyceride rich LDL and HDL. Hence, both LDL and HDL get reduced in

PATHOGENESIS OF DIABETIC DYSLIPIDEMIA



Effects of IR on lipid profile

High TGL levels
Low HDL levels
High proportion of small dense LDL particles

size. Epidemiological data suggest large buoyant LDL is less atherogenic than small dense LDL. Increased permeation of arterial endothelium, prolonged residence time of the particle in plasma with lower levels of antioxidants leads to the accumulation of lipids.[44]

Lipoprotein lipase effectively clears the dietary fat from the blood. But, lipoprotein lipase activity is greatly diminished in insulin deficiency or in insulin resistance and chylomicrons may be found 24 hours after last ingestion. Enormous accumulation of chylomicrons occurs in blood when this happens for a prolonged period.

Pancreatic lipase is activated locally and fatty acids are generated forming soaps (fatty acids generator) by detergent action of pancreatic lipase and more digestive enzymes are released, that lead to a more vicious damage. Hypertriglyceridemia is blood triglycerides level >150 mg/dl. The risk of pancreatitis increases above triglyceride level of 2000mg/dl. The production and release of VLDL is stimulated, attributing to hypertriglyceridemia resulting in low levels of high density lipoprotein (HDL) in the circulation.

Also, higher levels of VLDL lead to increased exchange of cholesterol ester and triglyceride between VLDL and low density lipoprotein (LDL), increasing the triglyceride content of LDL and making them more susceptible to degradation by hepatic lipase, leading to formation of small dense LDL particles. The lipid profile in type2 DM individual is characterised by high triglyceride levels, low HDL cholesterol levels, and normal levels of LDL cholesterol, with relative increase in the number of highly atherogenic small

dense LDL particles. This '**diabetic dyslipidemia'** is one of the major factors underlying the increased incidence of atherosclerotic cardiovascular disease (ASCVD) in type2 DM.[45]

In adipose tissue defective insulin action leads to excessive breakdown of triglycerides and formation of Non–Esterified Fatty Acids (**NEFA).** High levels of NEFA can stimulate gluconeogenesis in liver and can reduce insulin stimulated glucose uptake by tissues.

According to guidelines of ADA and American Heart Association, the target lipid values in diabetic individuals (age >40 years) **without cardiovascular** disease should be as follows: LDL 100 mg/dl (< 2.6 mmol/L); HDL 40 mg/dl (>1 mmol/L in men) and 50 mg/dl (>1.3 mmol/L) in women; and triglycerides150 mg/dl (<1.7 mmol/L). In patients >40 years, the ADA recommends addition of a statin, regardless of the LDL level in patients with CHD and those without CHD, but who have CHD risk factors[46].

If the patient is **known to have CHD**, the ADA recommends an LDL goal of70 mg/dl (<1.8 mmol/L) as an "option". Older studies with fibrates increased the efficacy, but recent trials have not shown a benefit of this class of agents.

HYPERTENSION

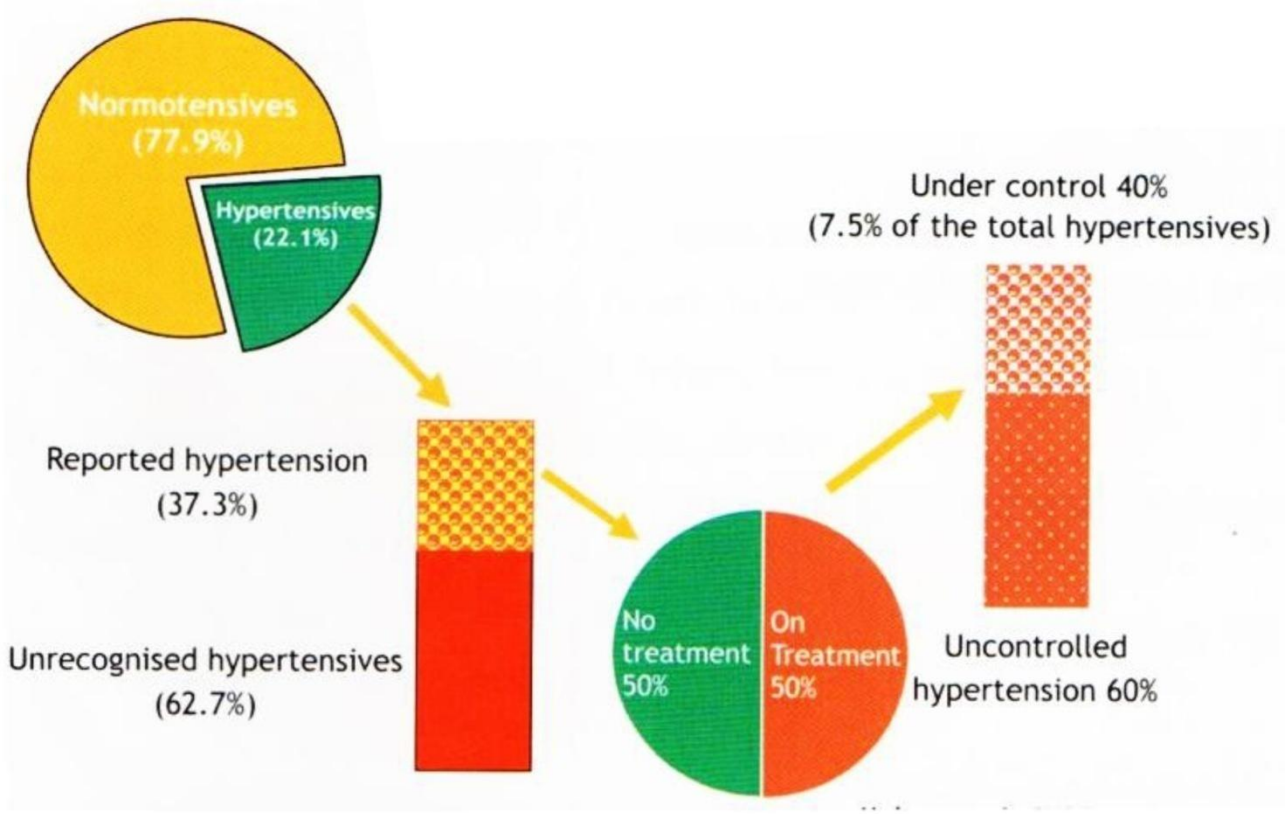
Hypertension is the most common co-morbid condition present along with diabetes. It is a major risk factor for both microvascular and macrovascular complications of diabetes. The prevalence of hypertension is 1.5-2 times greater in patients with diabetes compared to non diabetic patients. The prevalence of hypertension in India is estimated to be as high as 30%.most worrying is that "Rule of halves" is applicable to diabetes which states that 50% of hypertension is unaware that they have hypertension, 50% of known hypertensive's take treatment and only 50% who take treatment are under control[47].

National Health and Nutrition and Examination Survey **(NHANES)** 1999 – 2000, states that 43% women and 31% of men with diabetes had high blood pressure. Incidence of hypertension is increased approximately by 50% in diabetes and it is both due to insulin resistance and hyperinsulinemia.

Hypertension forms an important risk factor for cardiovascular disease including coronary artery disease, cardiac failure, cerebrovascular accidents and peripheral vascular disease. According to Framingham heart study when the SBP is increased by 20 mmHg there is a chance of 56% increased risk for cardiacfailure as proposed by **Adler et al**[48]

Hypertension in patients with diabetes, compared to those without diabetes, has unique features such as increased salt sensitivity, volume expansion, loss of nocturnal dipping of blood pressure and pulse, increased

PREVALENCE OF HYPERTENSION IN SOUTH INDIA -The rule of halves is still valid



propensity to proteinuria, orthostatic hypotension, and isolated systolic hypertension.

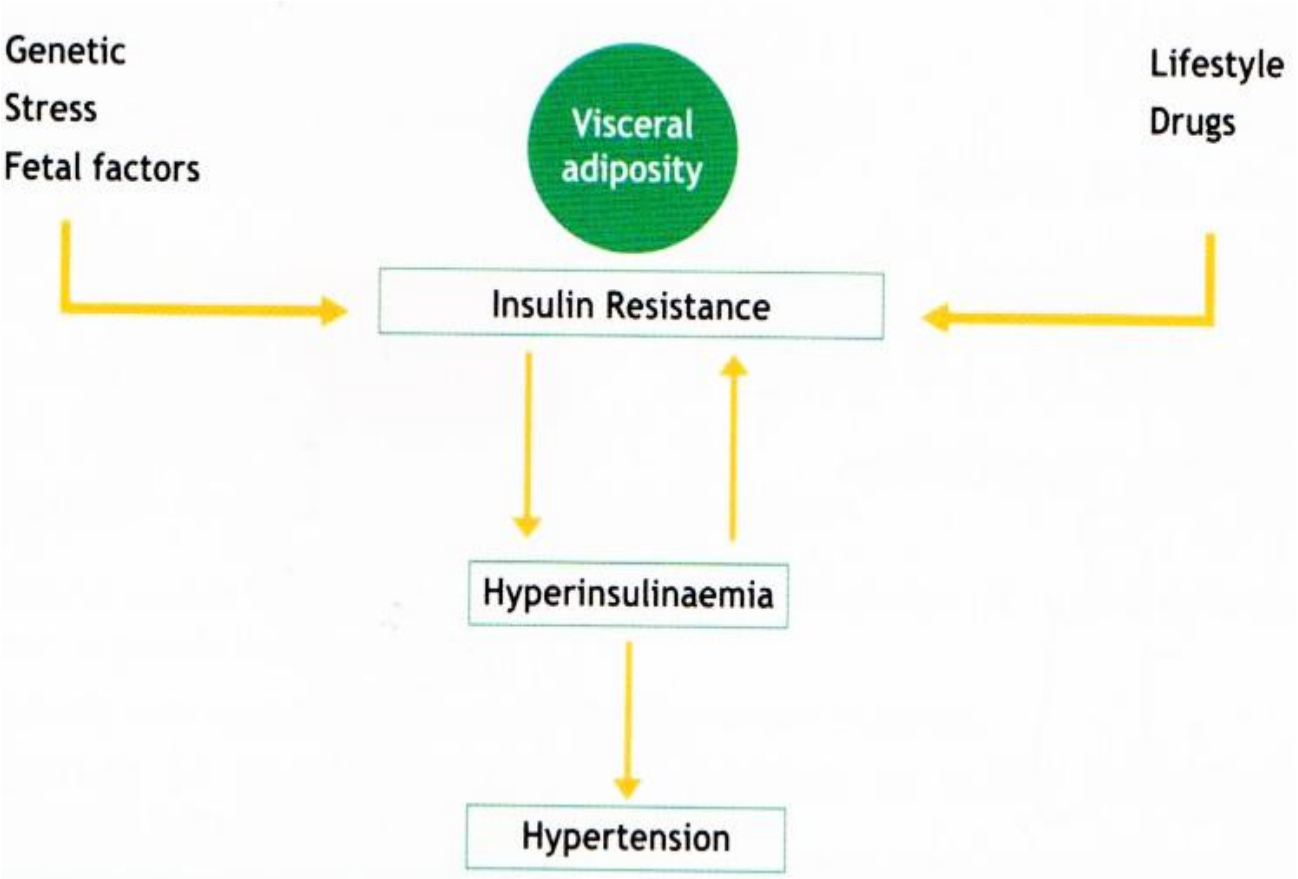
Pathophysiology of hypertension in diabetes

Hypertension and diabetes may be founded in the co-segregation of genes predisposing to both hypertension and diabetes. Fat and carbohydrate, influences the activity of the sympathetic nervous system. Premature rise in systolic blood pressure in people with diabetes associated with premature widening of pulse pressure. This has been attributed to various mechanisms including an age related decline in endothelial dysfunction and progressive accumulation of AGE's.

Ageing, mechanical strain, activation of the sympathetic nervous system and the rennin angiotensin-aldosterone system, along with glucose concentrations may play a role in promoting vascular matrix accumulation.

Increased arterial stiffness has profound effects on blood pressure and increases the vascular load on heart and the coupling efficiency between the heart and vasculature. This premature stiffing of the aorta in diabetes explains the earlier development of systolic hypertension and a wide pulse pressure in diabetic subjects when compared with non diabetic population. It also renders the systolic pressure more difficult to control with drug therapy.

PATHO PHYSIOLOGY OF HYPERTENSION



Staging of hypertension[49]

Joint national committee report VII **(JNCVII**): classification of hypertension (adults >18 years of age).

	Systolic BP(mmHg)	Diastolic BP(mmHg)
Normal	<120	<80
Prehypertension	120-139	80-89
Stage1 hypertension	140-159	90-99
Stage2 hypertension	≥160	≥100

-Systolic BP >140 mmHg is a more important risk factor for CVD than diastolic BP.

-The risk of CVD doubles with every increase of 20/10mmHg beginning at 115/75mmHg.

SMOKING

The combination of type 2 diabetes and smoking speeds up the rate of complications especially microvascular and macrovascular disease. Smoking with diabetes increases the risk of stroke, heart attack, peripheral vascular disease, kidney failure and diabetic eye disease.

Smoking leads to narrowing of blood vessels and damages the blood vessel walls. Nicotine causes constriction of blood vessels and release of hormones such as catecholamine which increases the blood pressure. Blood pressure in diabetes is hard to control because of accelerated blood vessel

damage. Smoking places additional strain on the body's ability to control blood pressure and smoking increases the insulin resistance.

Effects of smoking related to diabetes[50]

- Increased heart rate
- Increased LDL (bad) cholesterol.
- Reduced HDL cholesterol
- Raised blood pressure
- Increased platelets –blood is more viscous and likely to clot
- Atherosclerosis- narrowing of the arteries
- Increased insulin resistance.

OBESITY

Obesity has been described as the major pandemic of the 21st century. 30% of the population in most developed countries is obese. Studies from various parts of India indicate that 20-50% of the urban population is either obese or overweight. As more and more individuals and family break out of the grip of poverty, the prevalence rates of obesity can also be expected to go up.

Obesity is defined as an excessive accumulation of fat in adipose tissues, leading to medical illness, which occurs when there is an **imbalance** between "**energy intake**" and "**energy expenditure**".

OBESITY

Energy expenditure are determined by

(i) Thermogenic effect of food

(ii) Physical activity

(iii) Basal metabolic rate

The susceptibility of an individual to become obese in response to environmental factors is influenced by specific genes. Adipose tissue, muscle, liver, regulatory centre of the brain all play an important role in obesity.

BMI represents relationship between weight and height. More than BMI specific distribution of fat whether central or abdominal obesity is more important.

BMI ASSOCIATED DISEASE RISK AND WHO CLASSIFICATION OF OBESITY

	Obesity class	BMI(kg/m2)	Risk
Underweight		<18.5	Increased
Normal		18.5-24.9	Normal
Overweight		25-29.9	Increased
Obesity	I	30-34.9	High
	II	35-39.9	Very high
Morbid obesity	III	>40	Extremely high

Additional risk: (i) waist circumference > 90 cms in men and > 80 cms in women

(ii) Poor aerobic fitness

(iii) More than 5 kg weight gain between 18-20 years of age

(iv) Descent of Southeast Asia

INDIAN CLASSIFICATION OF OBESITY[51]

BMI(Kg/m²)	Classification
18.5-22.9	Normal
23.0-24.9	Overweight
25.0-29.9	Obese

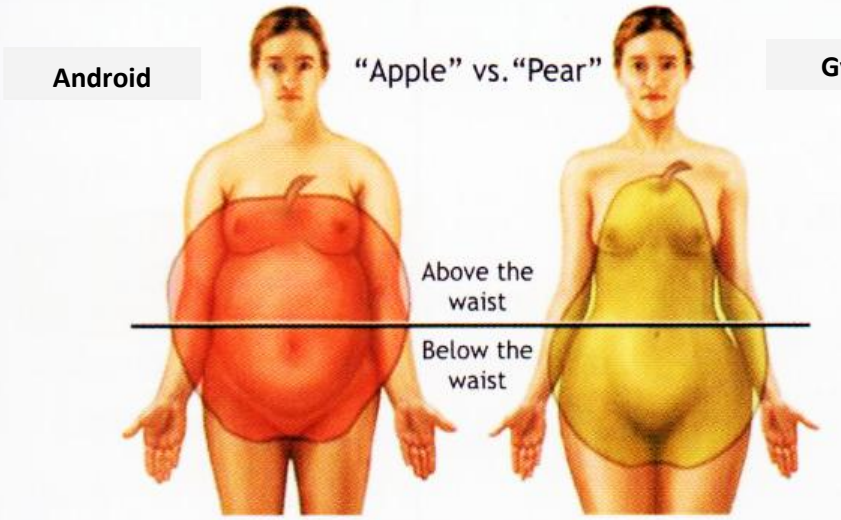
Obesity affects each and every organ and in particular, it is a major cardiovascular risk factor. The risk factor for obesity includes hypertension, diabetes, lipid abnormality, fibrinogen excess and markers of inflammation. The hall mark of cardio-metabolic syndrome is insulin resistance.

Types of obesity[52]

Android or central or visceral obesity-body resembles the **shape** of an **apple**. The fat gets accumulated mainly above the waist.

Gynoid obesity- the body resembles the **shape** of a **pear** i.e. the fat gets accumulated below the waist.

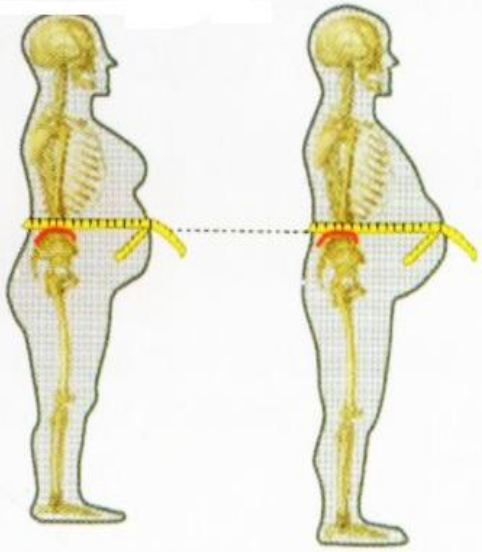
TWO VERY DIFFERENT TYPES OF OBESITY



The apple shape indicates increased visceral adiposity, which is an important risk factor for cardiovascular disease

BMI does not differentiate between these two types of obesity

Waist Circumference has been shown to be a good surrogate marker of abdominal obesity



In India, values >90 cm in males and >80 cm in females can be considered abnormal

Android or central obesity is strongly associated with the metabolic syndrome with the metabolic syndrome and increased risk of CVD. **BMI cannot differentiate the two types of obesity**

Use of WC or WHR is therefore recommended for diagnosis of central obesity. From convenient point of view, WC is preferred. For **Indians**, the cut-off of WC was kept at **90 cm (males), 80 cm (females).**

In spite of having lower body weight and BMI than their western counterparts, Asian Indians have found to have more type2 diabetes. This paradox has led to the concept of the "Asian Indian phenotype". As for the given body weight, Asian Indians have higher waist circumference, higher visceral obesity, more insulin resistance and more diabetes than their Caucasian counterparts. That is, the "**thin fat Indian**" may be physically lean but he is metabolically obese.

Diabetes and obesity

Obesity particularly visceral obesity is a strong risk for type 2 diabetes. Increased depots of visceral adipose tissue lead to increased delivery of free fatty acids to the liver, which lead to the development of insulin resistance in this organ.

Unlike subcutaneous fat, visceral fat is metabolically active which releases a variety of adipocytokines and inflammatory mediators which cause further reduction in insulin sensitivity. High levels of FFA can also inhibit insulin secretion from the pancreas leading to "**lipotoxicity"**.

STUDIES RELATED TO MICROALBUMINURIA

According to **John et al**[53] study male sex, old age, longer duration of diabetes, poor glycemic control, and raised blood pressure were risk factors for microalbuminuria.

Vijay et al[54]**.,** showed that duration of diabetes, systolic and diastolic blood pressure, age of the patient, serum creatinine with proteinuria are the risk factors. The association of glycemic control with microalbuminuria has been established by various studies.

Jafer et al[55]**,** .2009 have shown that strong association exists between microalbuminuria and major ECG changes and importance of screening Indo-Asian subjects for microalbuminuria in subjects with hypertension, diabetes, obesity and tobacco users for unmasking underlying CVD.

Gupta et al[56]reported that microalbuminuria is associated with raised HbA_1C. HbA_1C is increased when duration is increased or in uncontrolled diabetes. So these individuals have microalbuminuria positive.

UK based population study showed that microalbuminuria and gross proteinuria predicted future episodes of CHD with hazards ratios of 1.36 and 1.59 respectively **(Yuyun et al,)** [57].

STUDIES RELATED TO INFLAMMATION

Epidemiological studies begins to examine the hypothesis "elevated baseline inflammation predicts risk of coronary vascular disease. Various studies have shown the risk predictors of coronary heart disease are total leukocyte count (**Wheeler et al, 2004),** C-reactive protein,leucocyte count (**Danesh et al)**[58]and fibrinogen (**FSC 2005**).

Full blood count including red cell count, white cell count, C-reactive protein, lipid profile, including HDL, LDL, triglycerides, HbA_1C,are all positively correlated with increasing cardiovascular disease in type2 DM (**Kumar and Clark)**[59]**.**

Elevated basal levels of some mediators like cytokines such as interleukin -6 (IL-6) or tumour necrosis factor , soluble adhesion molecules and downstream acute phase reactants such as CRP, fibrinogen and serum amyloid A (SAA) are associated with elevated risk of cardiovascular events(**Libby et al,)**[60]**..**

Tumour necrosis factor and interleukin 6 (IL-6) are produced by adipocyte produces an acute phase response, that leads to an increased production of CRP, a sensitive marker of low grade systemic inflammation **(Hernández et al)**[61]**.**

STUDIES RELATED TO CARDIOVASCULAR RISK AND DIABETES

Henock Ambachew et al [62], have shown that diabetic patients are prone for increase in LDL and total cholesterol than other lipid abnormalities. This finding of dyslipidemia play an important role increasing the risk of developing cardiovascular risk the study also echoed the need of incorporating serum lipid profile as routine investigation to minimize complications. dyslipidemia and therefore cardiovascular risk.

Christos Kalofoutis et al[63] have shown that type 2 diabetes, metabolic syndrome, and insulin resistance are associated with cardiovascular mortality and morbidity. Targeting multiple cardiovascular risk factors offers best CVD outcome and. He also demonstrated low grade inflammation exists in these individuals leading to atherosclerosis.

Joanna Pollak et al[64] studies evidenced that microalbuminuria is associated with cardiovascular events and mortality. So screening for microalbuminuria with the sensitive and reliable methods available has clinical importance as that of blood pressure and lipid profile.

Seema Basi et al[65] study had concluded that the presence of albuminuria, a powerful predictor of cardiovascular risk in diabetes and hypertensive individuals. Reducing the level of albuminuria decreases risk of cardiovascular and renal outcomes.

The study done by **Gerstein et al[66]** have shown that microalbuminuria is an independent risk factor for cardiovascular events like cardiac failure and a cause of mortality in people with previous history of CHF.

King et al[67]have shown that CRP levels are increased in non diabetics with insulin resistance, in type 2 diabetes, and in type 1 diabetic individuals. Obesity rather than hyperglycemia contributes to elevated CRP levels. A proinflammatory state prevails in the prediabetics, 5 years before the actual onset of diabetes. This contributes to atherosclerosis and cardiovascular risk.

Desouza et al[68]studies concluded even the hypoglycemia occurring during the strict control of diabetes leads to the inflammatory activation and increase in counter regulatory hormones leading to macrovascular complications.

Pedicino et al[69] studies have shown that immunity and inflammation plays a key role in the pathogenesis of atherosclerosis and its complications. Immune responses in endothelial activation, plaque development, and rupture, as demonstrated by the increased levels of inflammatory markers or certain lymphocyte population and the risk of occurrence of cardiovascular morbidity and mortality.

Schultz et al[70]have demonstrated in his studies that BMI have an effect in the development of cardiovascular events. This association was subsequently confirmed in 2 separate cohorts of women. These findings provide evidence that the association between dietary factors and risk of 2 diabetes may be mediated in part by inflammation and endothelial dysfunction.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study design:

It is a cross sectional study.

Place of study:

The study was conducted in Tirunelveli medical college hospital, Tirunelveli.

Collaborating department:

Department of Medicine, Tirunelveli medical college, Tirunelveli.

Department of Biochemistry, Tirunelveli medical college, Tirunelveli.

Department of Pathology, Tirunelveli medical college, Tirunelveli.

Period of study:

6 months (December 2014-may 2015).

Study subjects:

200 diabetic individual (age **35-55** years), both men and women.

Inclusion criteria:

About 200 type 2 diabetic individuals of both men and women between 35-55 years were included.

Exclusion criteria:

- Type 1 diabetic individuals
- Known malignant disease
- After vigorous exercise
- History of heart failure
- Urinary tract infection
- Liver cirrhosis
- Haematological diseases
- Advanced renal dysfunction(serum creatinine >2.0mg/dl)
- Those taking medications like steroid and anti allergy agents.

Materials used for the study:

1. Proforma : To obtain detailed history, to record the anthropometric measurements of subjects, and clinical findings.
2. To detect Microalbuminuria from early morning urine sample using Semi auto analyser (Model : chem5x from Erba manheim).
3. To find Total Leucocyte count, differential count evaluated using semi autoanalyser (Model: Sysmex KX-21,Transasia Sr.No.KX-B8608 from Erba Manheim)
4. Fasting Blood Glucose using Semi auto analyser (Model: Chem5x from Erba Manheim).
5. HbA_1 C using Semi auto analyser (Model: Chem5x from Erba Manheim).

6. Lipid profile done using full auto analyser (Model:Multi XL V2014.03B EM 360 from Erba Maheim)
7. ECG was taken (Model: EDAN SE-1) machine.

METHODOLOGY

The study was initiated with the approval of Institutional ethical committee. The study was carried out after explaining the procedures in detail and getting written informed consent from all the subjects.

Measurement of blood pressure

Blood pressure was measured using mercury sphygmomanometer after 30 minutes of rest (**Pickering et al., 2005).** Blood pressure **≥140/90 mmHg** was considered as hypertensives.

Measurement of anthropometric indices:

Height:

Using stadiometer vertical height was measured in centimetres to the nearest 0.5 centimetres.

Weight:

Weight in kilograms was recorded using a portable standard weighing machine.

Body mass index:

Body mass index was calculated using the **Quetlet's** formula.

MEASUREMENT OF WAIST CIRCUMFERENCE

BMI =weight (kg)/height (m2). Individuals with BMI ≥ 25(obese) were taken for the study.

BMI(Kg/m²)	Classification
18.5-22.9	Normal
23.0-24.9	Overweight
25.0-29.9	Obese

Waist circumference:

Waist circumference was measured using a nonelastic measuring tape. Reading was taken in the horizontal plane, midway between the inferior margin of the ribs and the superior border of the iliac crest, at the level of umbilicus.

Increased waist circumference was >80 cm (women), >90 cm (men).

Blood investigations

Under strict aseptic precautions, blood samples were collected from antecubital vein using 5 ml disposable syringes. Out of that, 3ml was put in the centrifuge tube and allowed to clot for 15 minutes then it was centrifuged for approximately 10 minutes at a centrifugal force of 1000 x g to 2000 x g and separated the serum in plain tube for biochemical tests**.** Remaining 2ml was put in EDTA tube, the blood was mixed gently and used for hematological tests.

TAKING BLOOD SAMPLE

Haematological test

Total leucocyte count and differential count were found out using semiauto analyser.

Results were interpreted as follows:[71]

Types of cell	**Normal count(cells/mm^3)**	**Percent(%)**
Total leucocyte count	4000-11000	100
Neutrophils	2000-7000	50-70
Eosinophils	50-500	1-4
Lymphocytes	1500-4000	20-40
monocytes	200-800	2-8

Total leucocyte count >7400 cells/mm^3 is considered as high normal for the study.

SEMI AUTO ANALYSER

(TOTAL LEUCOCYTE COUNT MEASUREMENT)

Biochemical tests:

Lipid profile:

Total cholesterol and triglycerides were determined using Standard enzymatic methods. HDL-C was measured by direct assay method. VLDL was calculated by dividing triglycerides by 5, and LDL was calculated by taking the difference of total Cholesterol and VLDL.

Results interpreted as follows [72]

LIPID PROFILE

PARAMETERS		**NORMAL VALUES(mg/dl)**	**INCREASED(mg/dl)**
Total cholesterol		**150 -250**	**>200**
Triglycerides		**<150**	**>150**
LDL		**<100**	**>100**
HDL	**MEN**	**<40**	**>40**
	WOMEN	**<50**	**>50**

Fasting blood sugar:

Subject was instructed not to eat or drink except water for 10-12 hours and the early morning blood samples were taken. Normal fasting blood sugar is **<126mg/dl** according to ADA[73].So values ≥ 126mg/dl was considered for the study.

FULL AUTO ANALYSER

(LIPID PROFILE)

SEMI AUTO ANALYSER

(MICRO ALBUMINURIA)

HbA_1C

HbA1C was measured by photoelectric colorimeter model XSYSOO54.

Normal value is**< 6.5%**[74]**.** So values ≥ 6.5% was considered high for the study.

Microalbuminuria:

The first morning urine sample was collected in disposable containers from diabetic patients. Microalbuminuria was measured by using semi-quantitative auto analyser using reagent.

Normal value of albumin excretion < 25 mg/litre

Microalbuminuria ≥ 25-200 mg/litre

Individuals with microalbuminuria level ≥ 25-200 mg/litre was considered as **microalbuminuria**, and individuals with <25mg/litre was considered as **normoalbuminuria**.

ECG:

A resting 12 lead ECG was recorded in supine position with EDAN SE-1 machine. These ECGs were read and coded on the basis of Minnesota code classification 4–1 or 4–2 or 5–1 or 5–2 or 6–1 or 6–2 or 7–1 or 7–2 or 8–1 or 8–3 and interpreted as present or absent.[75]

All the collected data were tabulated for individual cases in the form of master chart for 200 volunteers included in the study and they were divided into two groups. **Albumin excretion ≥ 25-200mg/l** as **microalbuminuria group** and albumin excretion **< 25mg/l** as **normoalbuminuria group**.

RECORDING ECG

RESULT ANALYSIS

RESULTS

STATISTICAL ANALYSIS

This information collected regarding all the selected subjects were recorded in a Master Chart. When the data was analysed it was found that 66 diabetic individuals had microalbuminuria, 55 individuals had increased Total leucocyte count and 48 individuals had changes in ECG out of 200 subjects.

All the data were analysed using SPSS Software Version 11.

Using this software range, frequencies, percentage, means standard deviations, chi square and ‘p’ values were calculated. Data analysed using **unpaired t test**. Correlation was calculated using **Spearman rho Correlation**. P value was used to assess the significance. P value < .05 was considered more significant.

Microsoft excel was used to create charts, graphs and tables.

TABLE 1

INCIDENCE OF MICROALBUMINURIA AMONG STUDY GROUP

	Microalbuminuria (≥ 25-200 mg/l)	%	Normoalbuminuria (< 25 mg/l)	%	Total
Male	**34**	**17**	**70**	**35**	**104**
Female	**32**	**16**	**64**	**32**	**96**
Total	**66**	**33**	**134**	**67**	**200**

Incidence of microalbuminuria among study group was 33%.

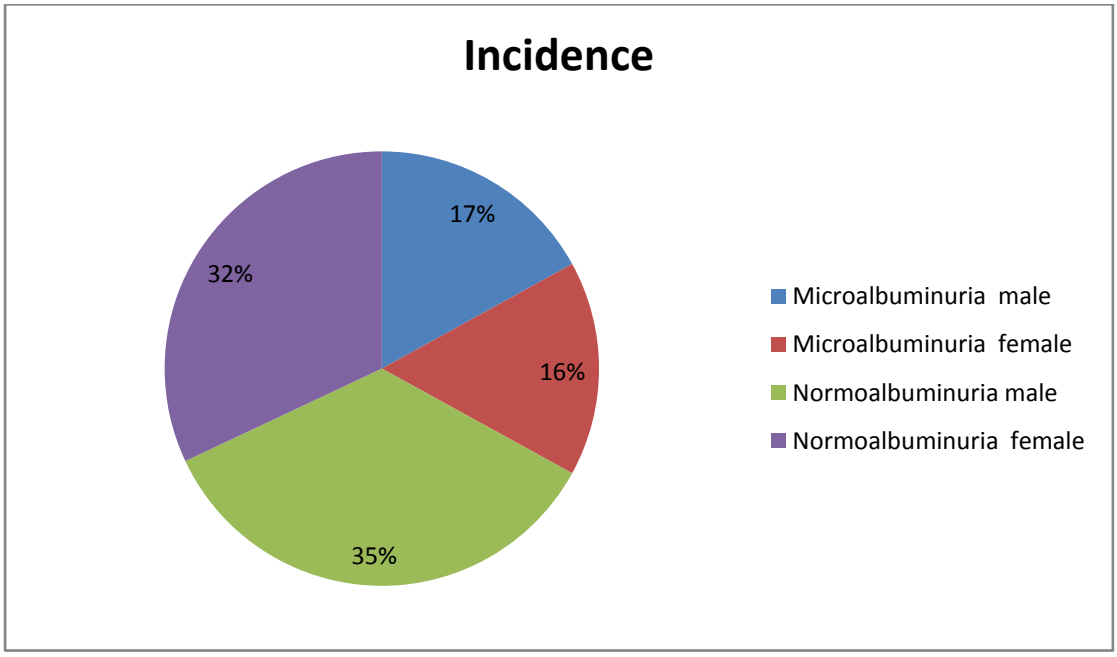


TABLE 2

CORRELATION BETWEEN MICROALBUMINURIA AND BMI

Group	BMI		P value
	Mean	SD	
Microalbuminuria (≥ 25-200 mg/l)	27.92	3.93	< .0001 Highly significant
Normoalbuminuria (< 25 mg/l)	25.79	3.05	

	Correlation coefficient	P value
BMI	0.195	0.006*

* Highly Significant

- There is significant correlation between microalbuminuria and BMI.
- BMI is increased in microalbuminuria group.

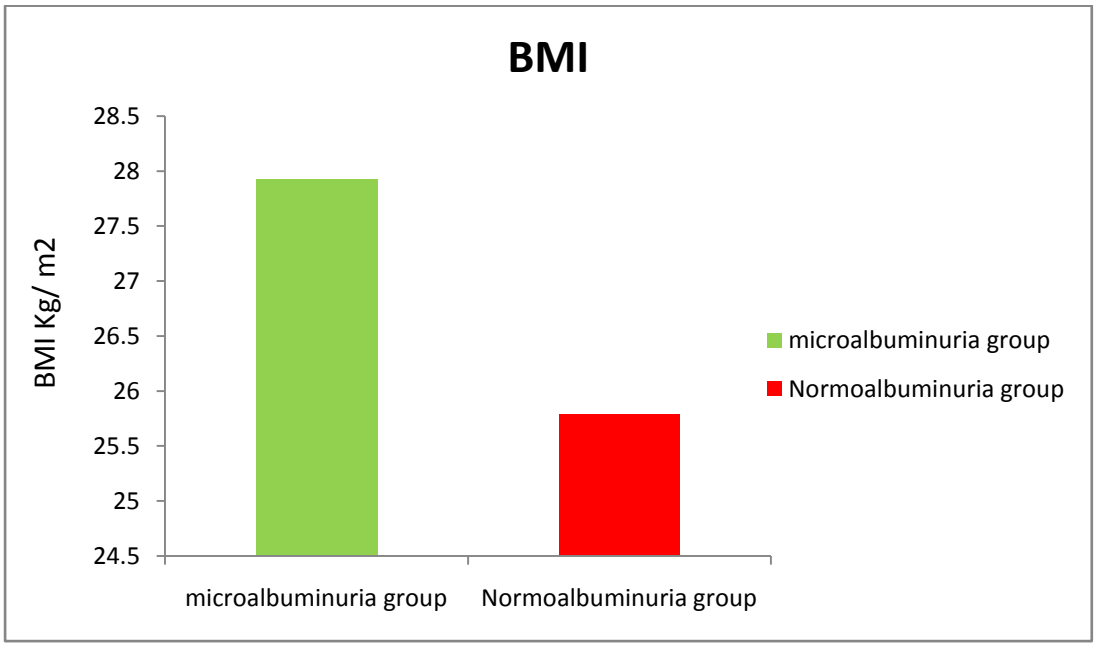


TABLE 3

CORRELATION BETWEEN MICROALBUMINURIA AND WAIST CIRCUMFERENCE

Group	Waist circumference		P value
	Mean	SD	
Microalbuminuria (≥ 25-200 mg/l)	95.02	6.72	0.002*
Normoalbuminuria (< 25 mg/l)	92.0	5.87	

	Correlation coefficient	P value
Waist Circumference	0.186	0.008*

* Highly Significant

- There is positive correlation between microalbuminuria and waist circumference.
- Waist circumference is increased in microalbuminuria group .

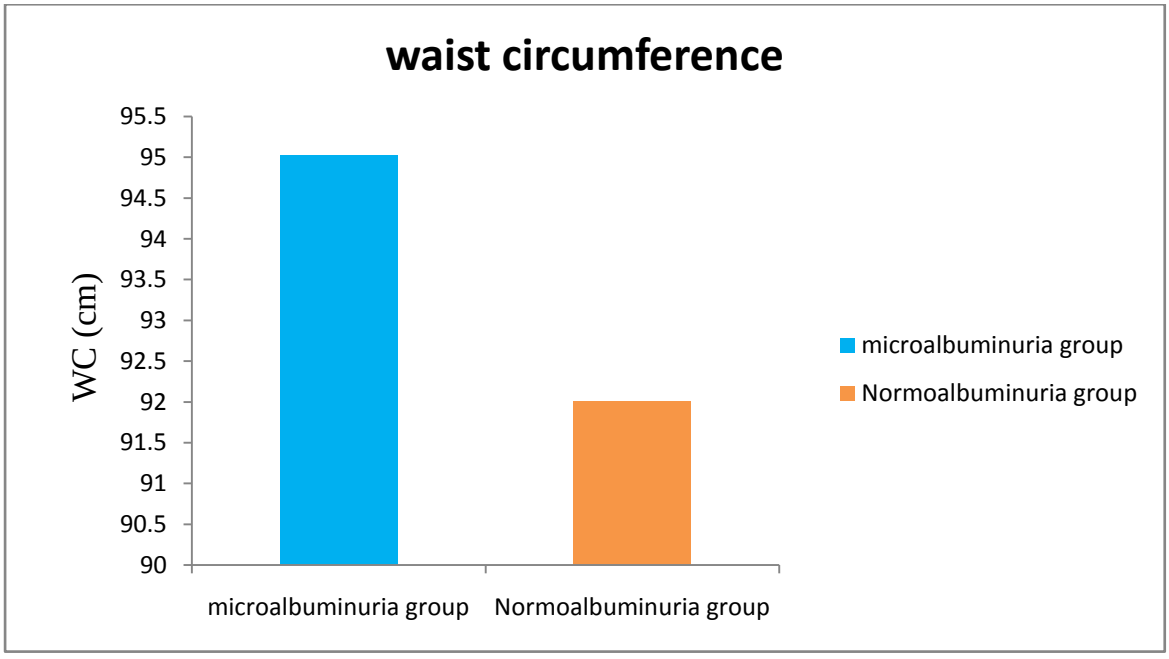


TABLE 4

ASSOCIATION BETWEEN MICROALBUMINURIA AND BP

Group	SYSTOLIC BP		DIASTOLIC BP	
	Mean	SD	mean	SD
Microalbuminuria (≥ 25-200 mg/l)	138.12	12.9	91.52	9.77
normoalbuminuria (< 25 mg/l)	121.37	16.72	76.313	11.66
P VALUE	<0.0001 Highly significant		<0.0001 Highly significant	

	Correlation coefficient	P value
SBP	0.485	<0.0001*
DBP	0.515	<0.0001*

* Highly Significant

- There is highly significant correlation between microalbuminuria , systolic as well as diastolic BP.
- Systolic and diastolic BP increased in microalbuminuria group.

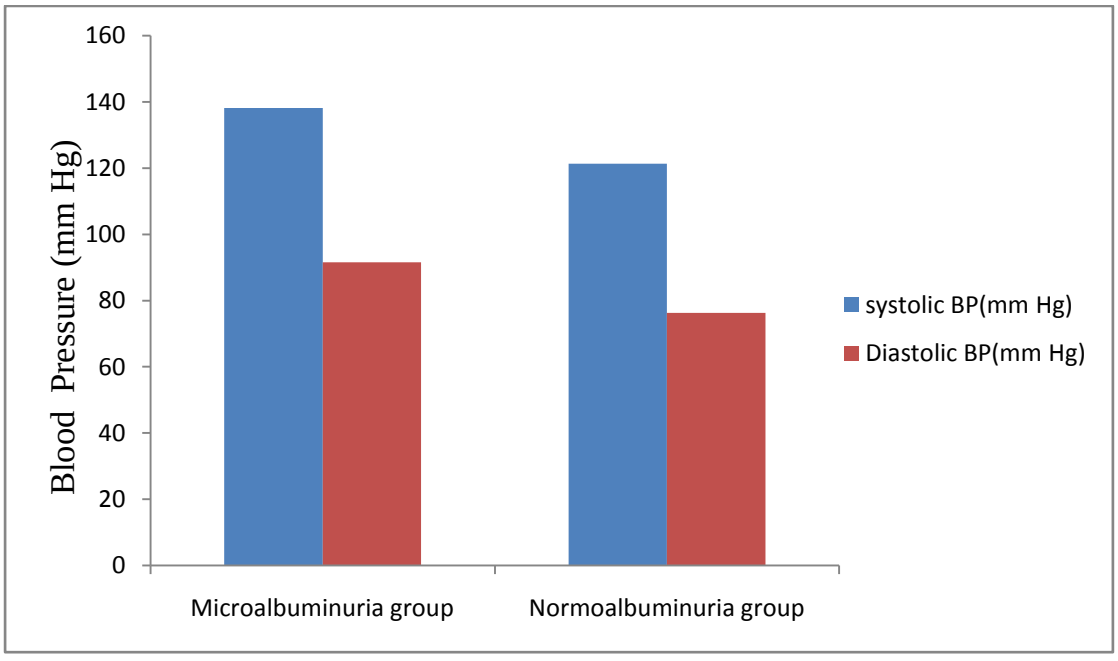


TABLE 5

CORRELATION BETWEEN MICROALBUMINURIA, TLC, DC

	Microalbuminuria (≥ 25 -200mg/l)		**Normoalbuminuria (< 25 mg/l)**		P VALUE
	Mean	**SD**	**Mean**	**SD**	
TLC	8596.97	1677.08	5625	1398.8	<0.0001*
NEUTROPHILS	68.01	9.47	61.32	7.57	<0.0001*
EOSINOPHILS	3.95	2.97	4.46	4.46	0.404
LYMPHOCYTES	18.72	6.80	28.29	8.64	<0.0001*
MONOCYTE	8.13	4.15	6.59	2.72	0.002

***HIGHLY SIGNIFICANT**

	Correlation coefficient	P value
Total leucocyte count	0.563	<0.0001*
Neutrophils	0.462	<0.0001*
Eosinophils	-0.067	0.348
Lymphocytes	-0.475	<0.0001*
Monocytes	0.073	0.304

* Highly Significant

- There is highly significant positive correlation between microalbuminuria , total leucocyte count and neutrophils
- .There is no correlation between microalbuminuria and eosinophil count.
- There is significant negative correlation between microalbuminuria and lymphocyte count. Individuals with positive microalbuminuria had decreased lymphocyte count.
- There is correlation between microalbuminuria and monocyte count, but not significant.

CORRELATION BETWEEN MICROALBUMINURIA, TLC, DC

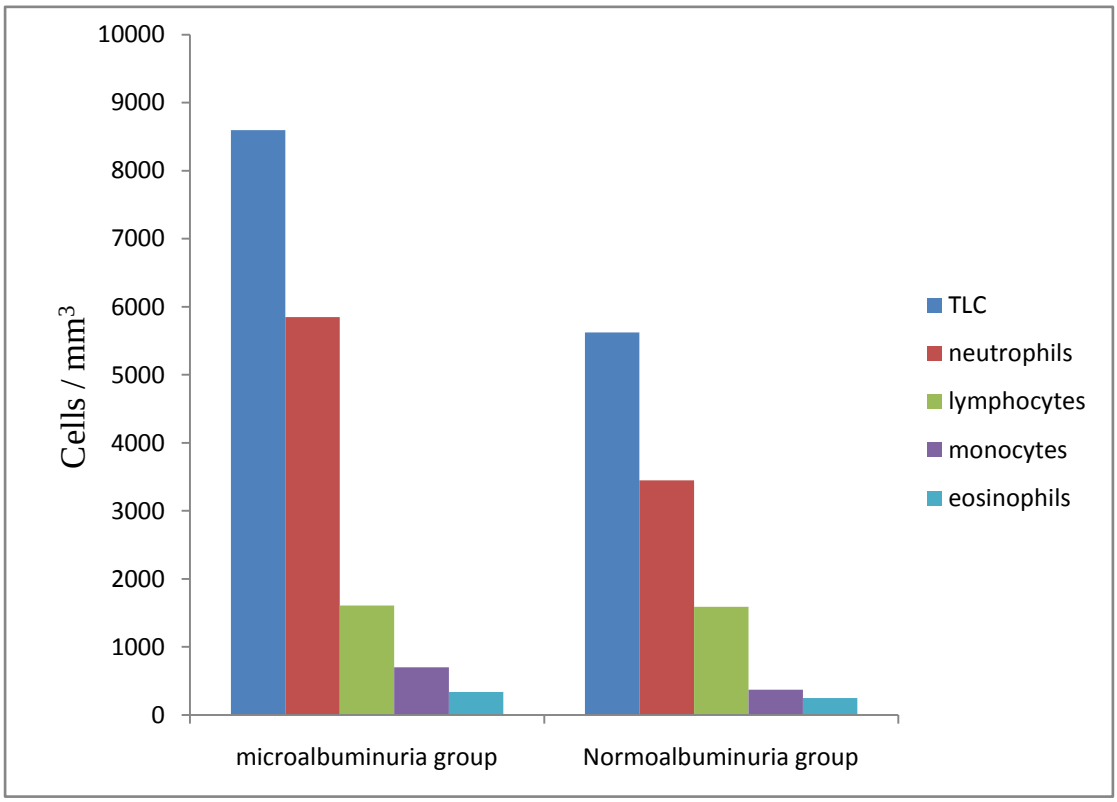


TABLE 6

CORRELATION BETWEEN MICROALBUMINURIA AND LIPID PROFILE

	Microalbuminuria (≥ 25 -200mg/l)		Normoalbuminuria (< 25 mg/l)		P VALUE
	Mean	SD	Mean	SD	
Total cholesterol	190.11	41.54	179.01	27.75	0.025
Triglycerides	190.61	30.95	156.96	29.73	<.0001*
LDL	99.74	8.84	96.91	12.99	0.112
HDL	43.17	5.33	47.72	5.40	<.0001*

	Correlation coefficient	P value
T.Cholesterol	0.023	0.744
Triglycerides	0.368	<0.0001*
LDL	0.237	0.001*
HDL	-0.316	<0.0001

* Highly Significant

- There is no significant association between microalbuminuria and total cholesterol.
- Significant correlation exists between microalbuminuria and triglycerides.
- Correlation between microalbuminuria and LDL level is not significant.
- There is significant correlation between microalbuminuria and HDL level.

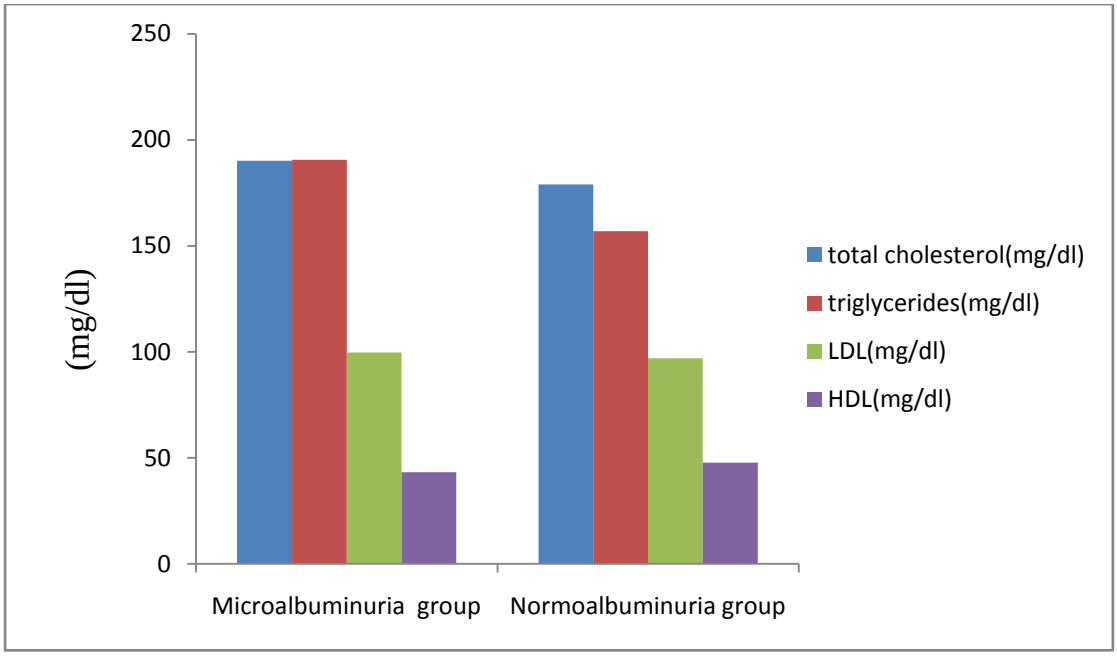


TABLE 7

ASSOCIATION BETWEEN MICROALBUMINURIA AND FBS

Group	FBS		P value
	Mean	SD	
Microalbuminuria (≥ 25-200 mg/l)	137.74	43.36	< .0001 Highly significant
Normoalbuminuria (< 25 mg/l)	99.06	18.76	

	Correlation coefficient	P value
FBS	0.478	<0.0001*

* Highly Significant

- There is highly significant correlation between microalbuminuria and FBS.
- FBS is above normal among the individuals in microalbuminuria group.

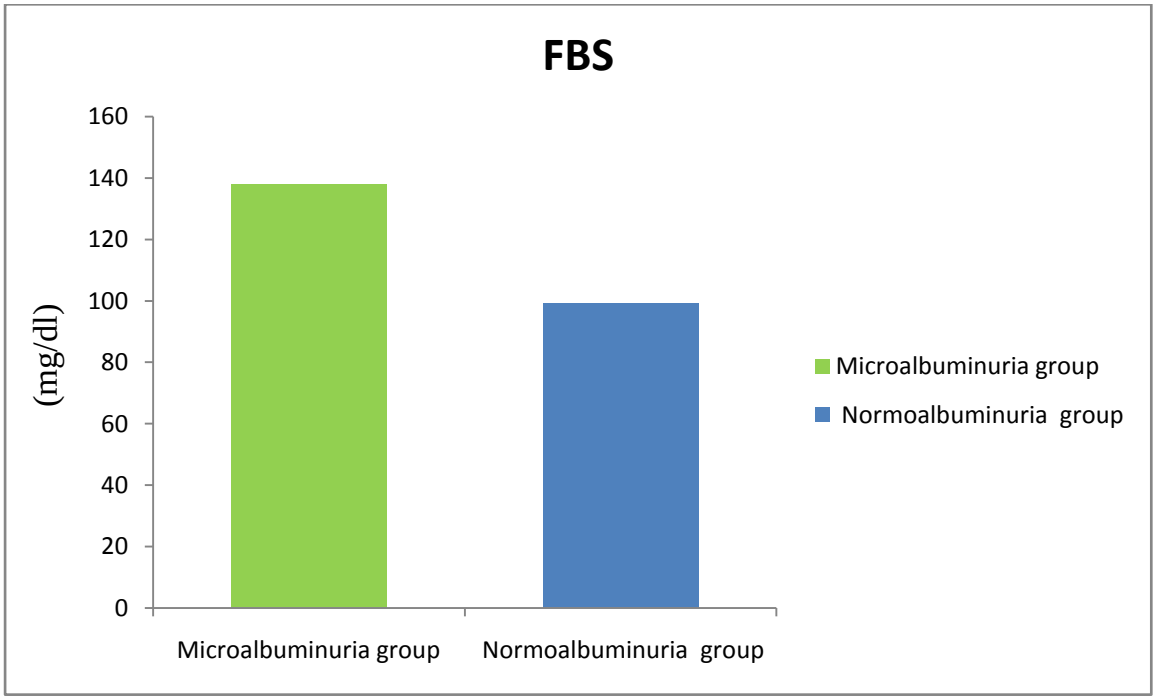


TABLE 8

ASSOCIATION BETWEEN MICROALBUMINURIA AND HbA $_1$C

Group	HbA_1C		P value
	Mean	SD	
Microalbuminuria (≥25-200mg/l)	7.86	0.86	< .0001 Highly significant
Normoalbuminuria(<25mg/l)	6.35	0.82	

	Correlation coefficient	P value
HbA1c	0.576	<0.0001*

* Highly Significant

- There is highly significant correlation between microalbuminuria and **HbA_1C**.
- **HbA_1C** value is increased in microalbuminuria group .

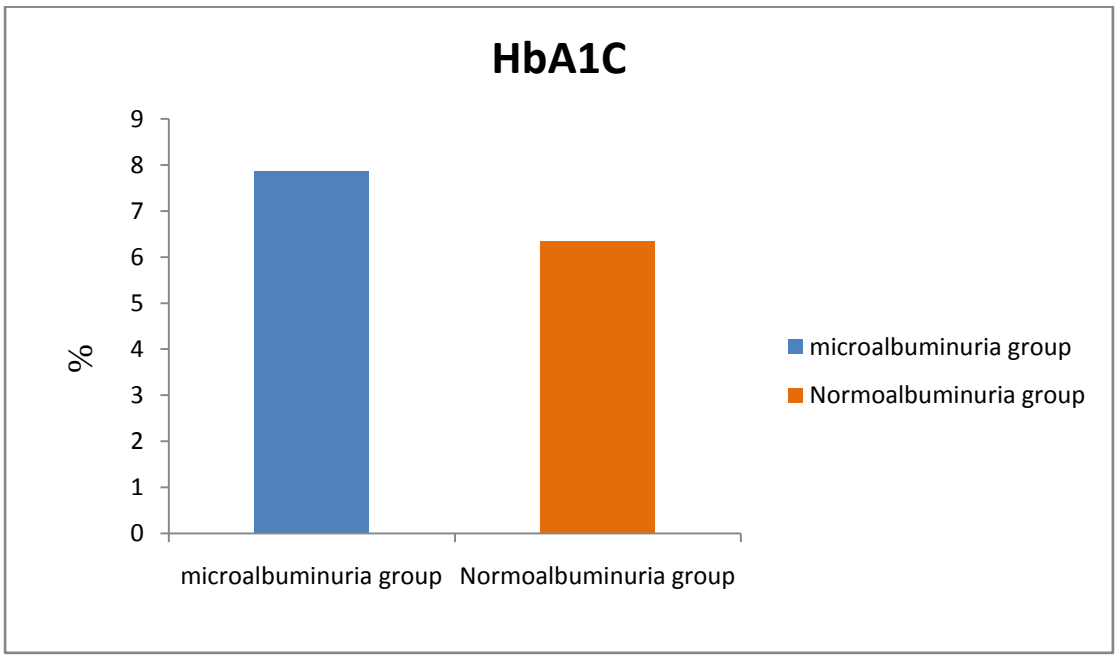


TABLE 9

ECG CHANGES IN THE STUDY GROUP

ECG CHANGES	Microalbuminuria (25-200mg/l)		Normoalbuminuria (<25mg/l)		**TOTAL**
	No.of.persons	**%**	No.of.persons	**%**	
PRESENT	29	15	11	6	40
ABSENT	37	19	123	62	160
TOTAL	66	34	134	68	200

	Correlation coefficient	P value
ECG	0.334	<0.0001*

* Highly Significant

- More number of persons in Normoalbuminuria group had no changes in ECG.

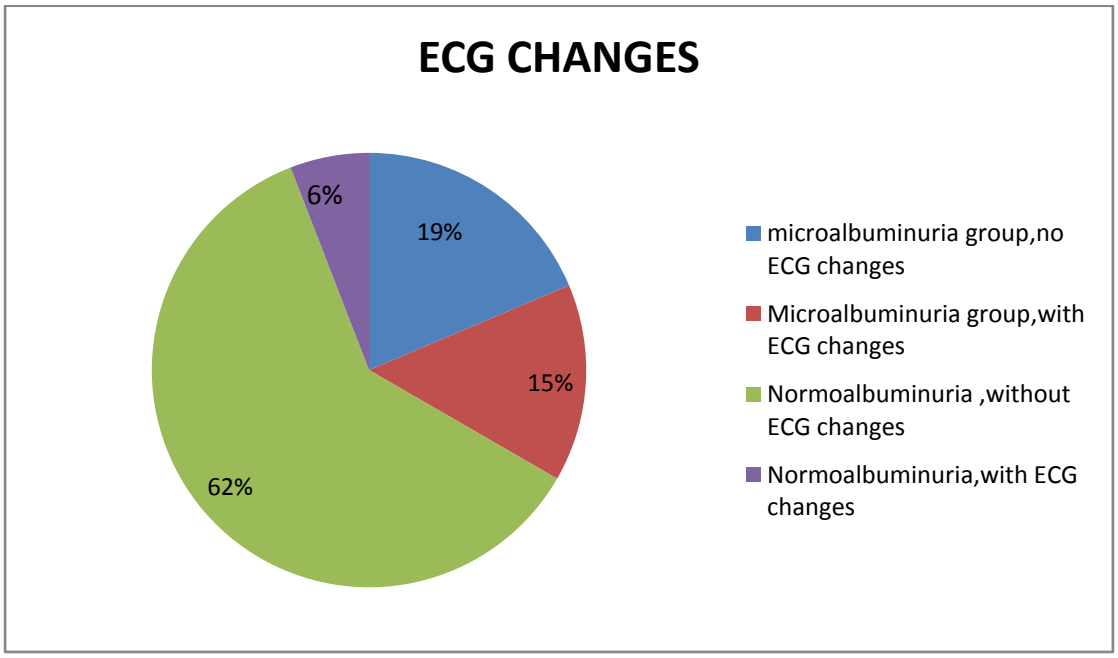


TABLE 10

RELATION BETWEEN MICROALBUMINURIA AND SMOKING HISTORY

SMOKING HISTORY	Microalbuminuria (≥25-200mg/l)		Normoalbuminuria (<25mg/l)		TOTAL
	No.of.person	%	No.of.persons	%	
PRESENT	24	12	38	19	72
ABSENT	42	21	96	48	138
TOTAL	66	33	134	67	200

- There is no significant correlation between microalbuminuria and smoking
- But more non smokers are normoalbuminuric.

	Correlation coefficient	P value
SMOKING	0.080	0.261

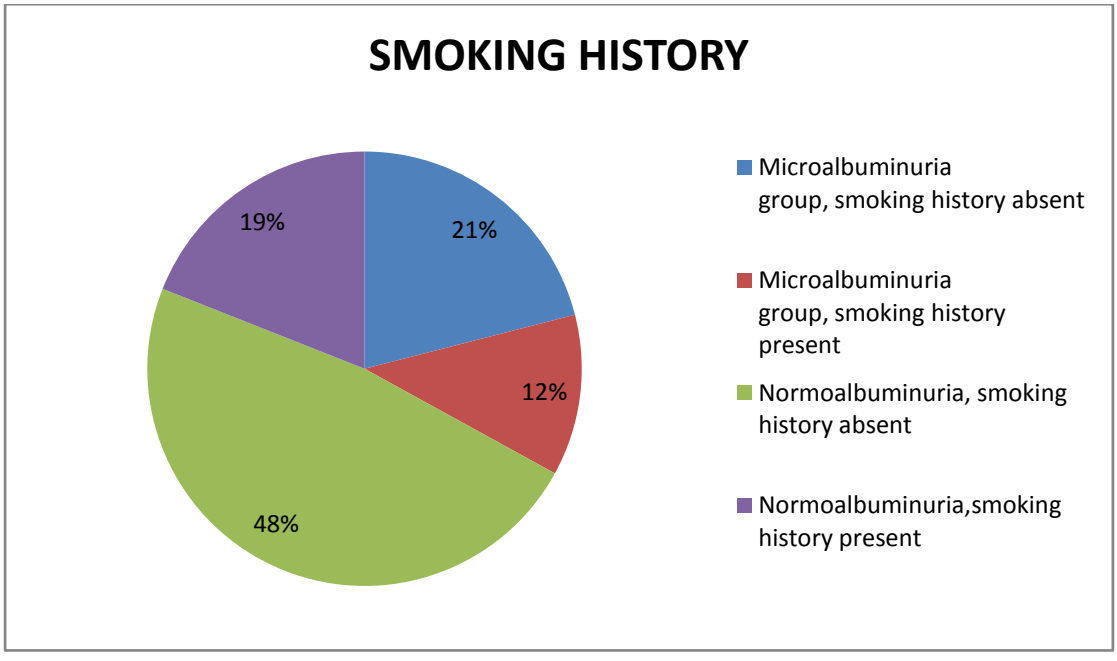
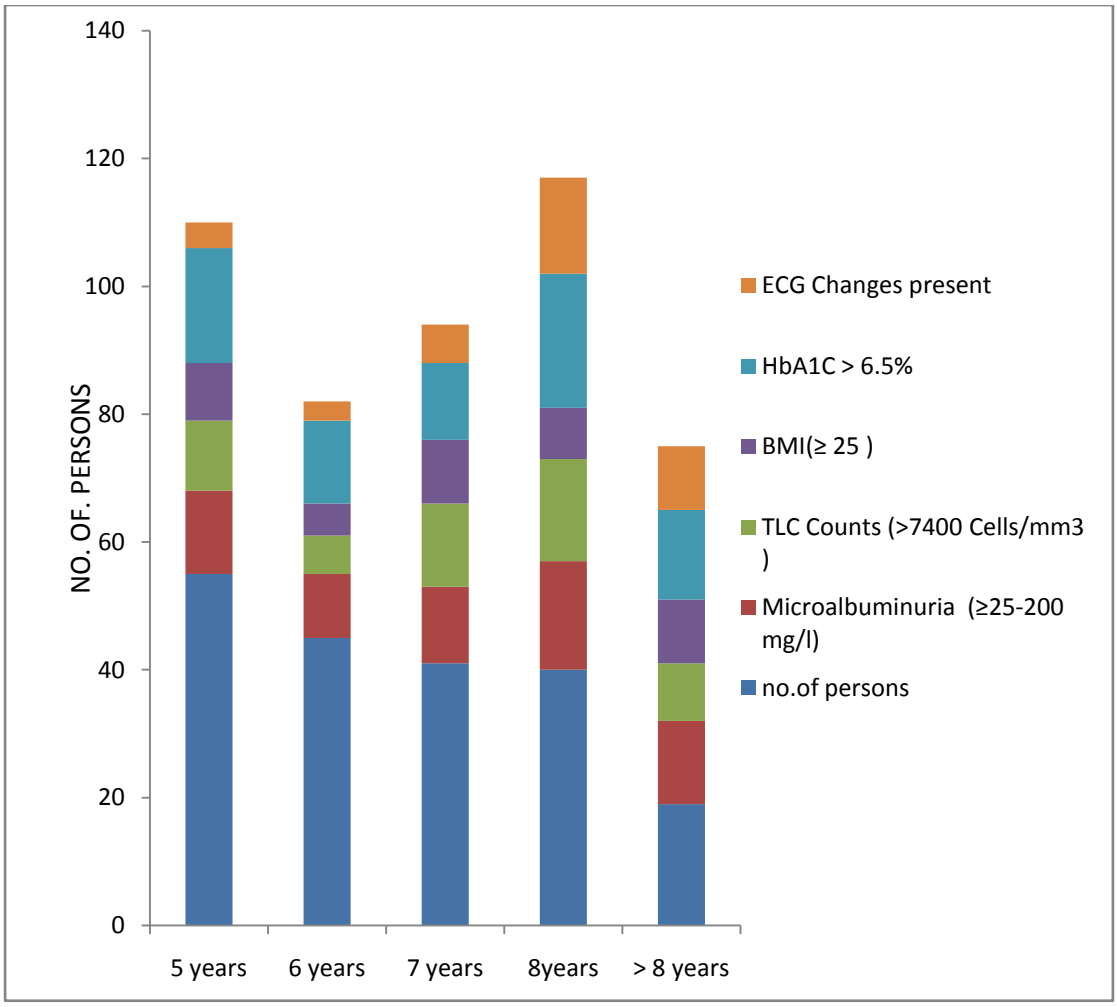


TABLE 11

COMPARISON OF PREVALENCE OF MICROALBUMINURIA, LEUCOCYTE COUNT, BMI, HBA1C, AND POSITIVE ECG CHANGES IN RELATION WITH DURATION OF DIABETES.

Duration (years)	**Persons N=200**	**Microalbuminuria (≥ 25-200 mg/l) N=66**	**TLC > 7400 Cells/ mm^3 (N=55)**	**BMI ≥ 25 N=42**	**HbA1C > 6.5 % N=78**	**ECG changes present N=48**
5	55 (27.5%)	13 (19.7%)	11 (20%)	9 (21.4%)	18(23%)	4(8.3%)
6	45 (22.5%)	10 (15.2%)	6 (10.9%)	5 (11.9%)	13(16.7%)	3(6.3%)
7	41 (20.5%)	12 (18.2%)	13 (23.6%)	10 (23.8%)	12(15.4%)	6(12.5%)
8	40 (20%)	17 (27.8%)	16 (29.1%)	8 (19%)	21(26.9%)	15(31.3%)
>8	19 (9.5%)	13 (19.7%)	9 (16.4%)	10 (23.8%)	14(17.9%)	10(20.8%)

COMPARISON OF PREVALENCE OF MICROALBUMINURIA, LEUCOCYTE COUNT, BMI, HBA1C, AND ECG CHANGES IN RELATION WITH DURATION OF DIABETES



DISCUSSION

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Investigations to find a suitable biomarker for inflammatory process in type 2 diabetes and its association with the complications have been underway for many years. Numerous biomarkers have been investigated. In this study we have investigated whether total leucocyte count can be added to diabetes control protocol as an early bedside routine investigation. Inflammatory process of the whole system can be reflected by total leucocyte count and this study was conducted to correlate the total leucocyte count, differential count, microalbuminuria and cardiovascular risk in type 2 diabetes mellitus.

The prevalence of microalbuminuria in type 2 DM in our study was 33%, which was similar to the study done by **Ahmedani et al** [76] at Karachi which was about 34%. In another study done by **Nevi Pasko et al** [77]in Tirana, the prevalence was 38%.The greater prevalence of microalbuminuria in the study done by Nevi Pasko et al may be due to greater duration of diabetes in patients selected. In our study there was no significant difference in the prevalence of microalbuminuria in both sexes.

Our study showed that patients with microalbuminuria group had higher BMI, and increased waist circumference. The mechanism responsible for this is that the adipose tissue not only act as an energy storage but also act as an endocrine organ and release various adipocytokines causing endothelial change leading to microalbuminuria.

In our study individuals with microalbuminuria had elevated systolic as well as diastolic blood pressure. This could be due to the endothelial dysfunction produced by microalbuminuria and causing development of atherosclerosis.

In our study, we found that patients with microalbuminuria had elevated total leucocyte count. This was due to activation of leucocytes by advanced glycation end products, oxidative stress and angiotensinII by hyperglycemia which produce changes in the endothelium resulting in microvascular complications. This result was similar to the study done by **Zaiden et al**[78]which showed that, elevated leucocyte count even though found in the normal range may have an effect on development of microalbuminuria. This might be due to activation of inflammatory process associated with diabetes.

In this study, microalbuminuria group with elevated leucocyte count had more ECG changes indicating the atherosclerotic changes produced by prolonged inflammation in the coronary vessels. **Chung et al** [79] found that peripheral WBC's, monocytes and neutrophil count increased in patients with the advancement of diabetic nephropathy. And the elevation in leucocyte count was associated with both micro and macrovascular complications, in diabetic individuals with microalbuminuria.

In another cross sectional study in 2004 by **Chan et al**[80] on 3776 Chinese diabetic patients in Hong Kong, it was demonstrated that higher leucocyte count even though in the normal range was independently related to both microvascular and macrovascular complications of diabetes and this

study also found a direct relationship between leucocyte count , BMI, and HbA_1C.

When differential count was considered in this study, microalbuminuria individuals had elevated neutrophil count, but not other counts especially lymphocyte count was in negative range. The mechanisms for this could be due to neutrophil influx from marrow storage and decreasing efflux from the blood stream.

Another theory is that neutrophil production is stimulated in bone marrow by low insulin levels in blood. The immune system of diabetic patients contains some receptors that induce inflammation in the blood vessels. In addition to other risk factors, chronic inflammatory responses can lead to diabetes complications by inducing massive endothelial injury and increase in some mediators. **Bjornson et al; collier et al** [81]both had quoted similar mechanisms in their study.

Leptin secretion from adipocytes is also increased by cortisol and insulin resistance and this leptin might be involved in increased leucocyte count by stimulating myeloid differentiation from human bone marrow CD34progenitors and can induce proliferation, differentiation, functional activation of hemopoietic cells as shown in studies done by **Gainsford et al**[82]

The study done by **Hanson et al**[83] **have** shown that inflammation plays a key role in atherosclerosis and coronary heart disease. Immune cells dominate early atherosclerotic lesions and lead to progression of the lesions and activation of inflammation lead to acute coronary syndromes.

Another study conducted in **Turin University** on 659 Italian patients by **cavalot et al**[84] showed that in patients with diabetes WBC count correlated with albumin excretion rate, one of the marker of subclinical inflammation associated with both insulin resistance and atherosclerosis.

In our study also there was a positive correlation between duration of diabetes, control of diabetes and cardiac changes. **Madjid et al**[85]had also demonstrated a correlation between leucocyte count and ischemic heart disease. Possible mechanisms include endothelial injury by proteolytic enzymes, vessel plugging, decreased perfusion, abnormal leucocyte aggregation, electrical instability, increased thrombus formation, increased leucocyte adhesion in coronary artery disease.

Jafar et al[86] studies associated microalbuminuria positive individuals had more ECG changes and therefore had higher cardiovascular risk.

In the previous studies incidence of coronary heart disease was 1.9 fold higher with WBC counts >7000cells/mm³ than in those with counts <4800 cells/mm³.**Twig et al**[87] study on Israeli army personnel states that with WBC counts >7000cells/mm³ had twice incidence of coronary heart disease as those with WBC count <5000cellsmm³.

Yutaka Takeda et al [88] had concluded in his studies that elevated WBC count even in normal range is associated with acute coronary syndrome. It was also suggested that elevated WBC count does not reflect acute inflammatory process but a chronic inflammation responsible for plaque vulnerability.

Our study results showed that individuals with history of smoking had elevated total leucocyte count, microalbuminuria, triglycerides and ECG changes. This might be due to the activation of immune system and inflammatory processes leading to the endothelial dysfunction and atherosclerosis.

Chong do lee et al[89] had supported our finding and he had demonstrated a dose response relationship between WBC count and cardiovascular disease mortality persisted for cigarette smoking.

Giuseppe schillaci et al[90] had a positive correlation with body weight, systolic blood pressure, cigarette smoking, fasting blood sugar, fasting insulin level and negative correlation with HDL , family income, alcohol consumption, physical activity or physical fitness.

In our study we had elevated fasting blood sugar and HbA_1C in microalbuminuria individuals and this was due to uncontrolled diabetes causing elevated HbA_1C and endothelial damage by advanced glycation end products.

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SUMMARY & CONCLUSION

SUMMARY AND CONCLUSION

- Diabetic individuals had a greater risk of developing both microvascular and macrovascular complications. Mortality due to diabetes is mainly because of cardiovascular abnormalities and cerebrovascular accidents. So if individuals prone for developing these complications are detected at an earlier date, it is possible to take necessary precautions to control the same. For that we need a simple and cost effective lab investigation that is done routinely.
- A correlation between total leucocyte count, differential count microalbuminuria and cardiovascular risk factors and ECG changes were investigated in 200 diabetic individuals.
- Multivariate analysis was done
- And it was found that 66 individuals had microalbuminuria, 54 had increased total leucocyte count and 48 had ECG changes.
- Smoking, positive family history of diabetes, increased BMI and increased waist circumference had elevated total leucocyte count, microalbuminuria, increased triglycerides, and increased blood pressure.
- Individuals with increased fasting blood sugar, HbA1C had elevated total leucocyte count, microalbuminuria and ECG changes.
- The results showed statistical significance.

- To conclude, since there exists a correlation between total leucocyte count, microalbuminuria and cardiovascular risk, total leucocyte count can also be used as a simple and cost effective method to detect the microvascular and macrovascular complications earlier thereby reducing the mortality and morbidity associated with diabetes.

FUTURE SCOPE OF STUDY

FUTURE SCOPE OF THE STUDY

- In the future we can involve a large number of subjects (>1000) including both male and female.
- We can also include acute phase reactants like C-reactive protein and fibrinogen to exclude acute infections.

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ANNEXURES

CORRELATION BETWEEN LEUCOCYTE COUNT, MICROALBUMINURIA AND CARDIOVASCULAR RISK IN TYPE 2 DIABETES MELLITUS

PROFORMA

Sl.no : **Income :**

Address :

Date :

Name :

Age :

Sex :

Personal history

1. Dietary history : Veg / Non-veg

2. Sleep duration : hrs. Regular / Iregular

3. Physical activity: Yes / No

4. Smoking history : Yes / No

5. Intake of alcohol :Yes / No

6. History of diabetes: Yes / No ,if yes duration :

7. History of hypertension: Yes / No ,if yes duration :

8.Family history of DM/HT/CAD/stroke : Yes / No

8. History of cardiac illness : Yes / No

9.Any other complaints :

CLINICAL EXAMINATION

Temperature : **Pulse : /min**

BP : mm Hg **RR : /min**

CVS :

RS :

ANTHROPOMETRIC MEASUREMENTS:

Height : ☐ **cm** **Weight :** ☐ **kg** **BMI:** ☐ **kg/m²**

Waist circumference : ☐ **cm**

HAEMATOLOGICAL PARAMETERS:

Total leucocyte count	Cells/mm³
Neutrophil count	Cells/mm^3
Eosinophil count	Cells/mm^3
Lymphocyte count	Cells/mm^3
Monocyte count	Cells/mm^3

BIO-CHEMICAL MEASUREMENTS:

Parameters	**values**
Blood cholesterol (mg/dl)	
Triglycerides (mg/dl)	
LDL (mg/dl)	
HDL (mg/dl)	
Fasting Blood sugar (mg/dl)	

ECG :

CONSENT : I hereby give my volunteer consent for the above study

Signature

CONSENT FORM

Dr. M. PRADEEPA, Post graduate student in the department of physiology, Tirunelveli Medical College, Tirunelveli is studying the Correlation between total Leucocyte count, Microalbuminuria and Cardiovascular risk in type2 diabetes mellitus-a cross sectional study. The test procedures are

1. Blood investigation (Total Leucocyte Count, Lipid Profile, HbA_1C, FBS)
2. Urine investigation (Microalbuminuria)
3. ECG
4. BP Measurement
5. Waist Measurement

The procedure was explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in the study. The data obtained may be used for research and other publication purpose.

Name:

Place:

Signature:

MASTER CHART

MASTER CHART (NORMOALBUMINURIA GROUP)

Sl.No	Name	Age	Sex	Smoking	DM (Duration in Years)	Family History	HT (CM)	WT (Kg)	BMI	WC (Cm)	BP (mm Hg)		Total count (cells/mm3	Differential Count (cells/mm3)				Lipid Profile				FBS (mg/dl)	Hb A1C (%)	Micro Albumina (mg/ltr)	ECG
											SYS	DBP		N	E	L	M	Total Cholesterol	Triglycerides	LDL	HDL				
1	Ponnuthai	49	F	A	6	A	140	55	28.1	86	100	70	6100	4148	122	1525	305	172	140	150	55	89	5.8	1.1	A
2	Kavitha	40	F	A	5	P	145	50	23.8	90	120	70	4800	3264	96	1200	240	168	140	94	45	90	6.8	1.3	A
3	Mathavan	52	M	A	7	P	165	60	22	90	120	70	5900	3658	177	1593	472	215	180	91	42	98	5.8	1.5	A
4	Syedalibeevi	37	F	A	7	A	160	55	21.5	80	110	70	5800	3712	116	1856	188	170	135	94	55	84	6.2	2	A
5	Solaiappan	50	M	A	5	P	162	70	26.7	98	140	86	4800	2976	96	1824	134	140	150	94	52	90	6.2	2.3	A
6	Shanaz	53	F	A	6	A	155	65	27.1	96	100	70	4800	3264	240	720	576	160	155	95	48	86	8.2	2.4	A
7	Sasikala	51	F	A	7	A	145	52	24.7	95	100	10	5500	3025	275	1870	330	220	145	99	48	83	5.8	2.4	A
8	Sathappan	38	M	A	2	A	155	55	22.9	92	130	80	5300	3498	212	1166	424	180	180	102	40	94	6.5	2.4	A
9	Raman	57	M	A	6	A	162	75	28.6	98	120	80	5700	3306	220	2052	114	190	145	96	46	140	6.2	2.4	P
10	Mariammal	52	F	A	5	P	150	48	21.3	80	116	90	4500	2790	225	1260	225	186	130	96	55	96	6.2	2.8	A
11	Paruthi	45	F	A	6	A	152	60	26	90	130	86	6700	3886	469	1675	670	195	186	93	48	120	5.8	2.8	A
12	Kaveri	52	F	A	6	P	148	45	20.5	83	120	72	4800	2640	1680	480	240	170	100	82	54	84	6.4	3.2	A
13	Antoniammal	52	F	A	8	A	145	62	29.5	98	110	70	4300	2322	172	1462	344	195	154	94	45	89	5.8	3.2	A
14	Poongothai	45	F	A	5	A	148	50	22.8	88	110	60	4800	2592	240	1728	240	180	150	89	40	89	7	3.2	P
15	Kayani	46	F	A	8	A	162	70	26.7	92	120	70	5300	3127	265	1378	530	205	195	90	46	97	4.8	3.3	A
16	Vijayasankar	48	M	A	8	A	175	80	26.1	92	130	70	5300	2862	318	1590	530	150	200	94	53	98	5.8	3.5	A
17	Andal	48	F	A	7	A	162	70	26.7	89	120	70	6200	3472	558	1550	558	180	215	98	52	96	5.8	4.2	A
18	K.Shanthi	45	F	A	7	A	150	45	24.4	81	100	60	4900	3332	98	1225	342	152	145	96	49	100	5.6	4.3	A
19	Rajan	42	M	P	7	P	155	60	25	98	120	80	4800	2928	96	1488	288	200	185	90	42	98	6.2	4.3	A
20	Shankar	51	M	P	8	A	160	70	27.3	98	130	80	4800	3024	240	624	528	180	145	85	42	98	4.1	4.4	A
21	Kandhi	48	M	A	6	P	170	78	27	96	130	60	4900	2940	147	1470	343	150	130	90	48	96	6.4	4.5	A
22	Nagarajan	48	M	P	5	A	152	62	26.8	96	120	86	6200	4340	310	1240	310	185	185	99	45	96	6.8	4.8	P
23	Mahalakshmi	45	F	A	6	A	155	60	25	88	110	70	5300	2915	159	1908	318	215	142	94	52	96	6.2	4.9	A
24	Pappammal	55	F	A	5	A	145	52	24.7	95	100	60	10100	7070	202	2525	303	250	146	94	50	124	6.8	5	A
25	Saraswathy	52	F	A	5	A	148	60	27.4	88	130	90	15200	12008	1620	912	760	252	180	98	42	130	8.2	5	A
26	Pockia selavn	43	M	A	7	A	168	65	26.4	98	100	80	4300	2666	172	1290	172	188	140	100	50	86	5.3	5.2	A
27	Deivarani	45	F	A	5	P	150	65	28.9	101	120	70	4800	3168	192	1152	288	180	140	104	51	92	6.2	5.7	A
28	Janaki	49	F	A	8	A	152	64	27.7	94	120	86	5300	2756	212	1908	424	185	130	100	50	78	5.4	5.8	A
29	Sudalai	48	M	P	6	A	158	78	31.2	94	130	76	4500	2520	90	1485	405	212	245	90	42	105	6.5	5.8	A
30	Ponnammal	50	F	A	5	A	160	58	22.7	82	130	80	4800	3360	96	1200	144	185	100	80	50	82	5.2	6	A
31	Ritta	55	M	A	10	P	162	54	30.1	90	130	80	4400	2640	88	1320	352	170	135	92	50	98	6.4	6	P
32	Kavitha	48	F	A	8	A	148	80	36.5	94	110	86	4900	2891	147	1519	343	165	145	94	40	96	5.9	6.2	A
33	Saraswathy	48	F	A	5	P	150	60	25.3	84	90	60	5100	2805	255	1734	306	160	155	196	54	86	6.3	6.4	A
34	Sankarammal	51	F	A	6	A	150	55	24.4	80	116	70	6300	4032	252	1512	504	180	142	90	52	88	6.8	7	A
35	Malar	47	F	A	7	A	145	50	23.8	86	110	80	5500	2860	220	1980	440	165	165	98	55	94	6.4	7.2	A
36	Kumar	50	M	P	6	A	170	78	27	92	130	80	5800	3712	232	1392	464	145	145	94	42	98	6.4	7.3	P
37	Pauldurai	55	M	P	10	A	156	70	28.8	86	100	60	6900	4416	276	2001	345	160	215	102	50	88	6.1	7.3	P
38	Senthil	46	M	P	8	P	165	72	26.4	104	150	70	4200	2310	126	1512	252	215	142	99	45	96	6.5	7.5	A
39	kadarali	39	M	A	5	P	165	68	23	94	110	70	5400	3024	216	1782	378	186	135	94	55	80	6.5	7.8	A
40	Kalangium	52	F	A	5	A	160	54	21.1	98	120	80	5200	3224	364	1196	416	215	144	95	52	98	6.2	8	P
41	Parvathy	40	F	A	5	A	142	52	25.8	84	120	70	6300	3969	252	1764	315	180	145	89	48	85	6.2	8	A
42	Mohammad	35	M	P	2	A	168	80	25	101	120	70	8000	4640	320	2400	640	185	145	92	53	85	6.8	8	A
43	Ismayil	50	M	P	5	A	148	52	23.7	94	120	80	5800	3944	116	1624	116	160	140	100	55	85	6.8	8	A
44	Meenakshi	48	F	A	8	A	142	55	27.3	88	150	90	4800	2880	144	1152	624	185	205	95	38	90	6.2	8.1	A

45	Ajmer	47	M	P	9	A	152	60	26	94	140	96	5200	2808	312	1716	364	125	194	98	43	80	7.4	8.1	A
46	Hari	45	M	P	5	A	160	72	28.1	90	110	80	5300	3127	265	1643	265	195	145	85	45	105	5.4	8.1	A
47	Kannan	54	M	P	7	A	164	80	29.7	94	150	110	9200	6440	184	2760	736	150	185	100	45	160	6.2	8.1	A
48	Baseera	50	F	A	5	A	148	62	28.3	98	160	100	5800	3596	174	1856	174	152	140	102	50	140	6.2	8.2	A
49	Saratha	46	F	A	8	A	156	65	26.7	92	110	80	5300	3392	159	689	1060	150	175	105	40	120	9.2	8.2	A
50	Kannan	52	M	P	5	P	168	82	29.1	102	120	80	5300	3180	212	1749	318	205	145	114	42	88	6.3	8.2	A
51	Romasubhu	43	M	A	5	A	168	72	25.5	96	120	70	8200	5904	164	2296	328	215	182	96	38	122	8.2	8.4	A
52	Vasan	52	M	P	8	A	170	80	27.7	101	140	100	6300	3528	315	2079	567	186	155	96	48	95	7	8.8	A
53	Lakshmi	50	F	A	6	A	152	62	26.8	82	120	80	5800	4988	174	1392	406	182	140	92	52	106	6.6	9	A
54	Kamatchi	54	F	A	6	P	148	60	27.4	98	120	80	4900	3234	196	1176	294	184	140	96	43	92	6.5	9	A
55	Ponprasad	45	M	A	5	A	165	60	22	95	132	80	4200	2268	210	1470	210	184	140	96	45	70	6.8	9	A
56	Bharathi	48	F	A	7	A	155	70	29.1	98	110	76	4800	2592	144	1728	336	220	185	101	52	94	5.9	9.1	A
57	Kuruvammal	54	F	A	8	A	168	75	26.6	86	110	70	5100	3468	102	1224	306	140	140	95	51	101	6.2	9.2	A
58	Fathimuthu	55	F	A	7	A	150	60	26.7	82	110	70	5300	3498	212	1272	318	155	135	94	52	92	6.3	9.2	A
59	Arafed	40	M	P	5	A	145	50	23.8	90	130	70	5800	3364	174	1914	348	185	215	94	40	96	5.8	9.2	A
60	Kumaravel	51	M	P	8	P	160	65	25.4	110	130	80	5300	2915	159	1908	318	215	142	95	42	160	6.5	9.3	A
61	Saravanan	46	M	P	6	P	165	73	26.8	90	120	60	4200	2310	126	1512	252	215	142	90	45	96	6.2	9.3	A
62	Ponmuthu	52	F	A	8	A	148	60	27.4	88	140	80	5800	3016	290	1972	522	192	136	94	48	95	5.3	9.4	A
63	Vani	42	F	A	6	P	158	65	26	94	100	70	4800	2592	528	1152	528	145	215	92	48	94	8.8	9.4	A
64	Malayammal	50	F	A	6	A	154	55	23.2	89	120	82	5800	3132	348	1740	580	186	142	80	53	92	5.6	10	A
65	Chelliah	54	M	P	7	A	158	50	20	92	120	70	5500	3025	825	1375	275	190	150	96	45	88	5	10	A
66	Pooranam	49	F	A	6	A	145	50	23.8	86	100	60	5500	3740	110	1430	220	184	135	94	50	82	5.8	10.15	A
67	Sathapan	43	M	P	5	P	162	65	24.8	91	130	80	5400	3348	162	1728	162	124	140	110	53	96	6.5	10.2	A
68	Karuppan	52	M	A	5	A	168	58	23	101	13.8	82	5300	3498	106	1378	318	200	142	101	43	85	5.5	11	A
69	Pappa	39	F	A	5	A	145	50	23.8	88	120	70	4800	2880	240	1344	336	140	190	94	42	115	6.2	11.3	A
70	Mookandi	40	M	P	5	A	168	75	20	101	130	88	6100	3782	183	1708	427	180	140	106	52	85	6.5	11.3	A
71	Karuppian	49	M	P	6	P	155	62	25.8	94	130	90	6900	4002	276	2070	552	160	155	98	50	94	5.2	11.3	A
72	Rajasekar	52	M	P	7	A	158	62	24.8	94	120	70	4300	2494	172	1290	344	145	125	82	50	99	3.1	11.3	A
73	Pari	48	M	P	6	A	155	60	25	90	130	90	5400	3132	216	1620	432	145	185	101	44	112	6.8	11.3	A
74	Muthukmar	52	M	P	7	A	155	60	25	105	110	70	8300	4814	332	2490	664	180	215	102	50	98	6.2	11.4	A
75	Poominathan	54	M	P	8	A	168	90	31.9	92	140	90	4800	2784	192	1440	384	180	225	101	40	96	8.1	11.4	A
76	Selvi	48	F	A	6	P	145	50	23.8	90	120	70	4700	2726	188	1410	376	145	190	92	35	140	5.6	11.8	A
77	Rajeswari	42	F	A	5	A	155	62	25.8	82	100	80	6200	3596	248	1860	496	180	145	92	52	96	5.3	12.2	A
78	Ibrahim	50	M	A	8	P	150	60	26.7	94	130	90	5400	3132	216	1620	432	213	140	90	50	96	7.8	12.3	A
79	Saraswathy	46	F	A	6	A	169	70	24.5	92	140	90	5700	3306	228	1710	456	215	138	98	52	98	7.2	12.4	A
80	Mahesh	50	M	A	5	P	155	80	33.3	104	130	70	4800	2784	192	1440	384	215	142	106	45	125	8.1	12.4	A
81	Sevugan	51	M	P	8	P	159	65	25.7	94	150	90	5300	3074	212	1590	424	176	160	104	52	99	6.6	12.4	A
82	Senthamarai	50	F	A	7	P	153	80	34.2	86	130	86	4800	2784	192	1440	384	180	215	90	40	84	5.8	12.5	A
83	Shermakani	59	F	A	8	A	142	45	22.3	89	110	70	5300	3074	212	1590	424	155	130	94	52	88	6.2	12.9	A
84	Geetha	48	F	A	6	A	158	68	27.2	88	130	60	4300	2494	172	1290	344	220	140	94	55	145	6.8	13	A
85	Alexandar	47	M	A	7	A	160	55	28.3	92	130	70	6200	3596	248	1860	496	180	140	94	45	90	7.3	13	A
86	Mathiyarasi	50	F	A	8	A	140	55	28.1	98	110	70	5400	3132	216	1620	432	135	195	97	40	96	6.5	13.1	A
87	Murugan	50	M	P	7	A	170	63	21.8	90	120	80	4800	2784	192	1440	384	145	142	115	42	85	6.8	13.1	A
88	Valliammal	54	F	A	5	A	155	50	20.8	88	130	70	4300	2494	172	1290	344	180	135	85	55	90	6.5	14	A
89	Kasilakshmi	45	F	A	5	P	152	56	24.2	88	110	70	4300	2494	172	1290	344	155	125	92	55	80	5.5	14	A
90	Mohammed rafi	52	M	A	6	A	170	75	26	94	130	80	8000	4640	320	2400	640	150	294	94	45	148	7.4	14	A
91	Michael	53	M	A	5	A	154	75	31.6	92	100	60	5300	3074	212	1590	424	124	140	105	48	96	6.8	14	A
92	Selvalakshmi	42	F	A	5	P	140	50	25.5	85	110	70	5500	3190	220	1650	440	155	170	90	55	94	5.8	14.2	A
93	Abbas	52	M	P	8	P	155	60	25	90	140	90	4800	2784	192	1440	384	170	155	105	52	98	6.8	14.2	A
94	Kamal	48	M	P	5	A	168	70	26.7	98	102	84	6200	3596	248	1860	496	150	130	101	42	96	6.4	14.3	A

95	Chinnakannan	52	M	P	7	P	160	82	32	96	120	86	5400	3132	216	1620	432	165	175	96	42	96	5.3	14.4	A
96	Shanmugathai	54	F	A	5	A	140	45	23	80	110	70	4500	2610	180	1350	360	182	140	92	50	9.2	6.5	15	P
97	Devadran	45	M	A	5	A	163	66	24.8	94	130	88	6400	3712	256	1920	512	185	156	92	50	90	5.5	15	A
98	Lakshmanan	49	M	A	5	A	175	78	25.5	92	130	70	5500	3190	220	1650	440	184	198	90	54	92	6.8	15.2	A
99	Paulsamy	41	M	A	5	A	172	65	22	96	140	80	5300	3074	212	1590	424	190	145	94	45	102	5.8	15.2	A
100	Rajamohan	50	M	P	8	A	155	60	22.7	93	130	80	4800	2784	192	1440	384	188	142	94	40	94	6.8	15.2	A
101	Valli	46	F	A	6	A	140	52	26.5	90	100	60	6800	3944	272	2040	544	140	150	89	52	90	5.6	15.3	A
102	Sundari	42	F	A	5	A	146	48	21.9	86	116	76	4800	2784	192	1440	384	250	156	96	52	80	6.5	16	P
103	Sundari	46	F	A	6	A	156	52	21.1	83	100	60	6200	3596	248	1860	496	186	140	82	52	78	5	16	A
104	Valliammal	50	F	A	5	P	158	55	22	82	130	80	5100	2958	204	1530	408	175	180	101	40	105	6.4	16	A
105	Retina raja	48	M	P	5	A	159	65	25.7	94	130	70	5300	3074	212	1590	424	186	142	94	44	86	6.3	16	A
106	Deepa	48	F	A	4	A	160	58	22.7	92	100	60	4500	2610	180	1350	360	165	125	94	52	88	6.6	16.1	A
107	Mareeswari	52	F	A	9	A	142	60	29.8	96	120	80	5900	3422	236	1770	472	145	205	99	50	89	6.5	16.1	A
108	Gomathy	54	F	A	6	A	152	45	19.5	85	140	80	5800	3364	232	1740	464	182	195	52	52	112	6.8	16.2	A
109	Chitra	38	F	A	5	P	148	60	27.4	86	140	90	4800	2784	192	1440	384	186	145	96	58	116	6.5	16.2	A
110	Muthu	45	M	P	6	A	150	55	24.4	92	150	100	6300	3654	252	1890	504	182	160	102	50	130	6.2	16.4	A
111	Ramachandran	52	M	A	10	A	175	76	24.8	91	140	90	4800	2784	192	1440	384	185	100	97	52	117	6.4	17	A
112	Vijaya	45	F	A	7	A	155	60	25	84	120	80	4300	2494	172	1290	344	145	185	88	30	120	7.9	17.1	A
113	Arivalagan	52	M	A	8	P	162	68	25.9	101	130	80	4800	2784	192	1440	384	185	146	98	34	92	6.4	17.2	A
114	Thevendran	52	M	A	6	A	163	60	22.6	100	120	86	7200	4176	288	2160	576	260	182	95	52	126	6.2	17.5	A
115	Angelin	50	F	A	7	A	152	65	28.1	95	130	70	4700	2726	188	1410	376	110	180	98	40	110	6.2	18	A
116	Vembu mariappar	46	M	A	6	A	152	50	21.6	92	130	70	7200	4176	288	2160	576	216	142	101	52	105	6.2	18	A
117	Sundarajan	50	M	P	6	P	164	72	26.8	94	110	70	6500	3770	260	1950	520	215	138	102	45	98	6.8	18.1	A
118	Avudaiappan	53	M	P	5	A	158	75	30	96	110	70	5100	2958	204	1530	408	220	135	96	58	82	5.4	18.1	A
119	Malathy	49	F	A	6	A	162	68	25.7	88	106	58	4800	2784	192	1440	384	180	180	100	50	92	6.4	18.2	A
120	Saraswathy	44	F	A	6	A	158	66	31.1	84	110	70	5100	2958	204	1530	408	175	130	98	52	80	6.2	18.2	A
121	Senguvan	48	M	A	6	A	175	68	22.8	90	130	70	4800	2784	192	1440	384	152	140	120	51	86	7.2	18.2	A
122	Balachandran	50	M	A	5	A	165	70	25.7	94	140	90	7200	4176	288	2160	576	195	154	145	48	125	6.4	18.2	A
123	Franklin	52	M	A	6	P	155	75	31.2	95	120	80	4800	2784	192	1440	384	220	140	104	48	125	6.3	19.1	A
124	Sankar	55	M	A	8	A	172	76	25.5	94	140	90	9200	5336	368	2760	736	196	135	89	40	132	6.5	19.3	P
125	Devandran	48	M	P	8	P	152	60	26	92	140	80	6500	3770	260	1950	520	182	145	99	52	116	7.8	20	A
126	Kuppusamy	55	M	P	10	A	170	82	28.4	98	130	80	9200	5336	368	2760	736	190	140	94	50	95	5	20	P
127	Balasubramanian	51	M	A	6	A	155	55	22.9	92	130	70	4700	2726	188	1410	376	180	140	98	42	94	6.5	20	A
128	Suresh	42	M	P	6	P	155	63	20.6	97	120	70	6700	3886	268	2010	536	180	140	94	40	102	6.7	20	A
129	Joseph	54	M	A	7	A	145	60	28.5	96	104	80	4300	2494	172	1290	344	186	145	99	46	130	6.2	20.2	A
130	Valliammal	54	F	A	7	A	155	60	25	88	140	80	4300	2494	172	1290	344	104	112	98	54	94	6.6	22	A
131	Petchmuthu	53	F	A	8	A	142	55	27.3	88	110	80	8100	4698	324	2430	648	180	152	102	50	110	7.1	22	A
132	Francis	52	M	A	7	A	162	82	31.2	102	150	100	4800	2784	192	1440	384	173	140	96	45	89	5.8	22	A
133	Lakshmi	46	F	A	6	P	144	54	26	88	118	70	5100	2958	204	1530	408	185	200	80	52	114	6.8	23.1	A
134	Siddha Serhmam	48	M	A	9	P	162	60	22.9	94	130	70	7100	4118	284	2130	568	150	185	93	50	120	6.8	23.2	A

MASTER CHART (MICROALBUMINURIA GROUP)

Sl.No	Name	Age	Sex	Smoking	DM (Duration in Years)	Family History	HT (CM)	WT (Kg)	BMI	WC (Cm)	BP (mm Hg)		Total count (cells/mm3	Differential Count (%)				Lipid Profile				FBS (mg/dl)	Hb A1C (%)	Micro Albumina (mg/ltr)	ECG
											SYS	DBP		N	E	L	M	Total Cholesterol	Triglycerides	LDL	HDL				
135	Sujatha	48	F	A	7	P	145	65	30.9	90	150	90	7400	4884	518	1332	666	225	186	94	45	186	6.2	25.2	A
136	Premkumar	54	M	P	9	A	166	72	26.1	88	150	100	9400	6204	658	1692	846	250	150	95	36	250	5.8	26.8	A
137	Subbiah	52	M	P	9	A	164	70	26	98	140	90	10700	7062	749	1926	963	205	245	103	42	272	8.4	27.7	P
138	Marithai	45	F	A	5	P	150	70	31.1	105	140	100	8800	5808	616	1584	792	189	215	82	45	125	8.4	29	A
139	Sudalai	52	M	P	12	P	168	92	32.6	105	150	100	7500	4950	525	1350	675	208	208	105	36	138	9.2	29.3	A
140	Ramaiah	53	M	A	8	A	158	80	26.6	100	140	96	8300	5478	581	1494	747	225	182	109	50	128	7.2	30	P
141	Vellammal	42	F	A	5	P	160	75	29.3	91	140	110	11000	7260	770	1980	990	200	200	108	45	126	8.2	31.5	A
142	Jospbine	55	F	A	6	P	145	55	26.2	90	130	90	7900	5214	553	1422	711	190	230	98	52	116	8.1	32	A
143	Pandaram	52	M	P	7	A	158	65	26	94	140	90	8300	5478	581	1494	747	250	186	92	46	125	7.8	32	P
144	Umamaheswari	53	F	A	7	A	145	70	33.3	102	130	100	8500	5610	595	1530	765	178	185	102	45	136	7.8	32.1	A
145	Sankari	52	F	A	8	A	140	50	25.5	89	110	70	10200	6732	714	1836	918	215	170	94	50	135	8.2	32.6	A
146	Mariammal	45	F	A	7	P	152	60	26	85	130	90	10000	6600	700	1800	900	163	106	104	48	130	8	33	A
147	velayutham	44	M	P	8	P	160	75	29.3	92	120	70	7800	5148	546	1404	702	225	185	98	45	125	7.5	34.1	P
148	Arunothaya	55	F	A	8	P	151	60	26.3	95	140	90	5700	3762	399	1026	513	286	180	92	42	130	8.1	34.7	P
149	Subbiah	45	M	A	5	P	163	80	25	94	150	90	8200	5412	574	1476	738	215	185	108	42	122	7.4	35	A
150	Mary	48	F	A	6	P	152	70	30.3	99	150	90	8200	5412	574	1476	738	154	185	98	40	125	7.4	36.5	P
151	Sindhu	50	F	A	7	P	159	62	24.5	98	150	100	8600	5676	602	1548	774	145	185	88	40	125	7.8	36.5	P
152	Rengammal	45	F	A	6	P	154	50	21.1	84	100	70	7800	5148	546	1404	702	190	120	95	56	90	5	38	A
153	Balkees	47	F	A	8	P	156	66	27.1	88	170	90	9300	6138	651	1674	837	216	186	99	45	122	7.9	40	A
154	Arunachalam	48	M	P	6	P	162	70	26.7	105	140	110	10300	6798	721	1854	927	152	190	105	40	130	7.9	40.2	A
155	Puthumani	55	M	A	6	P	150	65	28.9	94	150	100	6300	4158	441	1134	567	215	245	98	42	135	6.2	40.2	A
156	Udaiyappan	50	M	P	7	A	168	80	28.3	102	140	90	7500	4950	525	1350	675	185	215	98	34	180	8.4	40.3	P
157	Subbulakshmi	52	F	A	6	A	148	58	26.5	92	130	90	6300	4158	441	1134	567	176	203	96	50	125	7.4	42	A
158	Gurunathan	53	M	P	8	P	168	75	26.6	96	150	100	12000	7920	840	2160	1080	145	200	105	40	198	7.8	42.1	P
159	Arasi	52	F	A	7	A	152	68	29.4	90	150	90	8200	5412	574	1476	738	154	185	92	40	125	6.2	42.4	A
160	Subbiah	42	M	P	6	P	150	54	24	92	130	86	7400	4884	518	1332	666	190	146	86	45	80	8	42.5	A
161	Munigeswar	54	F	A	9	A	140	75	38.3	101	140	100	10200	6732	714	1836	918	146	205	94	42	240	7.8	42.8	P
162	Ravi	53	M	P	9	P	165	80	29.4	101	150	90	7800	5148	546	1404	702	180	215	105	40	152	7.8	43.2	P
163	Velumani	54	M	P	8	A	150	65	28.9	102	130	90	8200	5412	574	1476	738	140	152	98	40	98	7.2	43.2	P
164	Mahesh	50	M	A	5	P	155	55	22.9	94	130	96	9500	6270	665	1710	855	235	180	98	48	126	9.1	43.4	A
165	Parvathy	55	F	A	8	P	150	65	28.9	88	150	100	8600	5676	602	1548	774	180	215	92	45	140	8.5	45.1	P
166	Ramanan	55	M	P	5	A	170	90	31.1	101	130	80	8200	5412	574	1476	738	185	245	115	38	250	9.4	45.4	P
167	Kulamani	45	F	A	5	P	160	62	24.2	90	150	96	12400	8184	868	2232	1116	145	185	96	48	126	7.9	47.8	A
168	Chinnammal	54	F	A	5	P	152	60	26	84	120	70	8300	5478	581	1494	747	135	140	96	52	96	6.8	48	P
169	Mupidathi	48	F	A	8	A	148	62	28.3	88	140	96	9900	6534	693	1782	891	190	235	130	42	126	9	50.3	A
170	Muthumanikam	39	M	P	4	A	162	58	22.1	94	100	70	6100	4026	427	1098	549	160	185	94	50	92	6.2	51	A
171	Fatima	53	F	A	9	P	158	85	34	92	130	90	8500	5610	595	1530	765	160	205	120	38	145	8.8	52.1	P

172	Puthiyavan	51	M	A	7	P	164	82	30.5	96	150	110	9200	6072	644	1656	828	150	185	94	42	160	9	52.4	P
173	Vethamani	50	M	A	7	P	160	52	32	92	130	90	8500	5610	595	1530	765	184	140	110	42	90	7.8	53.9	A
174	alangaram	48	M	P	7	P	158	90	36.1	105	150	100	9200	6072	644	1656	828	145	185	108	34	215	9.1	54.2	P
175	Ramu	55	M	P	15	P	160	90	35.2	98	130	90	9400	6204	658	1692	846	160	160	100	40	150	8.3	58.5	A
176	Ulagammal	54	F	A	5	A	148	60	27.4	90	130	90	8100	5346	567	1458	729	140	182	96	51	90	8.1	59	A
177	Syed ali	52	M	P	7	P	152	59	25.5	98	130	80	9200	6072	644	1656	828	250	180	106	40	128	7.3	59	A
178	Gomathi	53	F	A	8	A	145	50	23.8	94	120	70	7500	4950	525	1350	675	125	185	100	54	105	8.2	60.1	P
179	Sankarammal	52	F	A	6	P	152	68	29.4	84	150	90	9100	6006	637	1638	819	220	180	90	42	115	7.5	62	A
180	Mariappan. G	45	M	P	8	P	170	100	34.6	100	140	90	8300	5478	581	1494	747	140	140	97	38	140	8.1	62.4	A
181	Sanharalingam	52	M	P	5	P	160	70	27.3	105	140	90	10200	6732	714	1836	918	270	190	96	39	128	8.5	62.6	P
182	Mariappan	45	M	P	8	P	172	90	30.4	100	140	90	11000	7260	770	1980	990	152	184	102	40	280	8.1	65	P
183	Sekar	50	M	P	9	P	168	90	31.9	108	140	90	8300	5478	581	1494	747	212	245	102	50	180	8.8	66.8	A
184	Lakshmi	50	F	A	8	A	145	60	28.5	101	130	90	4300	2838	301	774	387	255	160	101	45	116	8	69	A
185	Francis	55	M	A	5	P	165	56	20.6	92	110	70	5900	3894	413	1062	531	155	180	104	52	116	6.8	69	A
186	Kumar	43	M	P	8	A	155	62	22	98	130	90	8300	5478	581	1494	747	168	215	98	38	135	8.1	70	P
187	Ramya	55	F	A	6	A	145	50	23.8	94	160	90	5500	3630	385	990	495	150	215	92	42	120	8.1	70.2	A
188	Dhasammal	55	F	A	10	P	148	62	28.3	95	140	90	5700	3762	399	1026	513	280	162	105	48	86	8.2	73	P
189	Santha	42	F	A	5	P	165	60	22	82	150	110	8500	5610	595	1530	765	205	180	80	35	128	8.2	75	A
190	Bathirakali	46	F	A	7	P	145	60	28.5	95	130	90	10500	6930	735	1890	945	300	285	85	50	120	8.2	78	P
191	Kabilan	55	M	A	9	A	165	80	29.4	98	140	90	7800	5148	546	1404	702	180	215	92	35	152	6.2	80.2	A
192	Parvathy	50	F	A	5	P	162	75	28.6	98	130	90	11000	7260	770	1980	990	185	215	101	42	135	8	86	A
193	Vellammal	50	F	A	6	P	155	48	21.5	82	150	90	5300	3498	371	954	477	180	180	98	40	82	7.5	88.5	A
194	Gomathi	55	F	A	8	A	150	70	31.1	84	140	100	8500	5610	595	1530	765	180	220	103	38	128	7.6	91	A
195	Subbiah	54	M	P	8	P	165	59	21.7	94	150	100	8000	5280	560	1440	720	285	180	105	40	130	8.3	97	P
196	Raju	55	M	P	12	P	158	86	29	98	150	100	12800	8448	896	2304	1152	146	183	96	48	124	9.1	98	P
197	kandasamy	40	M	A	5	P	168	86	30.5	98	140	100	9200	6072	644	1656	828	180	210	110	42	80	7.8	99	A
198	Kalangium	52	F	A	8	P	153	60	25.6	84	140	100	9000	5940	630	1620	810	180	225	116	46	130	8.1	107	P
199	Ramalingam	55	M	P	10	P	130	62	36.2	105	136	90	9500	6270	665	1710	855	184	182	94	32	128	8.5	120	P
200	Ramlinga perium	55	M	A	10	P	150	70	23.8	110	150	100	10300	6798	721	1854	927	184	182	120	40	130	8.3	129	P

N - Neutrophils
E - Eosinophils
L - Lyphocytes
M - Monocytes
FBS - Fasting Blood Sugar
LDL - Low Density Lipoprotein
HDL- High Density Lipoprotein
ECG- Electro Cardiogram Changes
A- Absent
P - Present